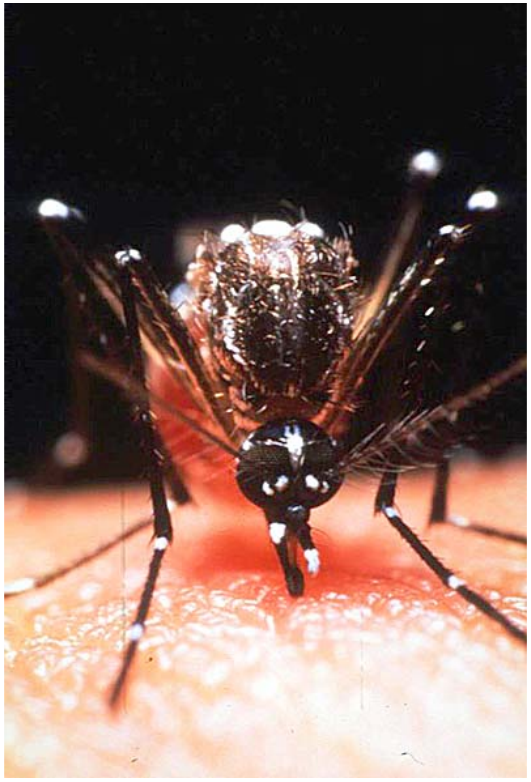


Dengue

Anna P. Durbin
Demystifying Medicine
January 13, 2015

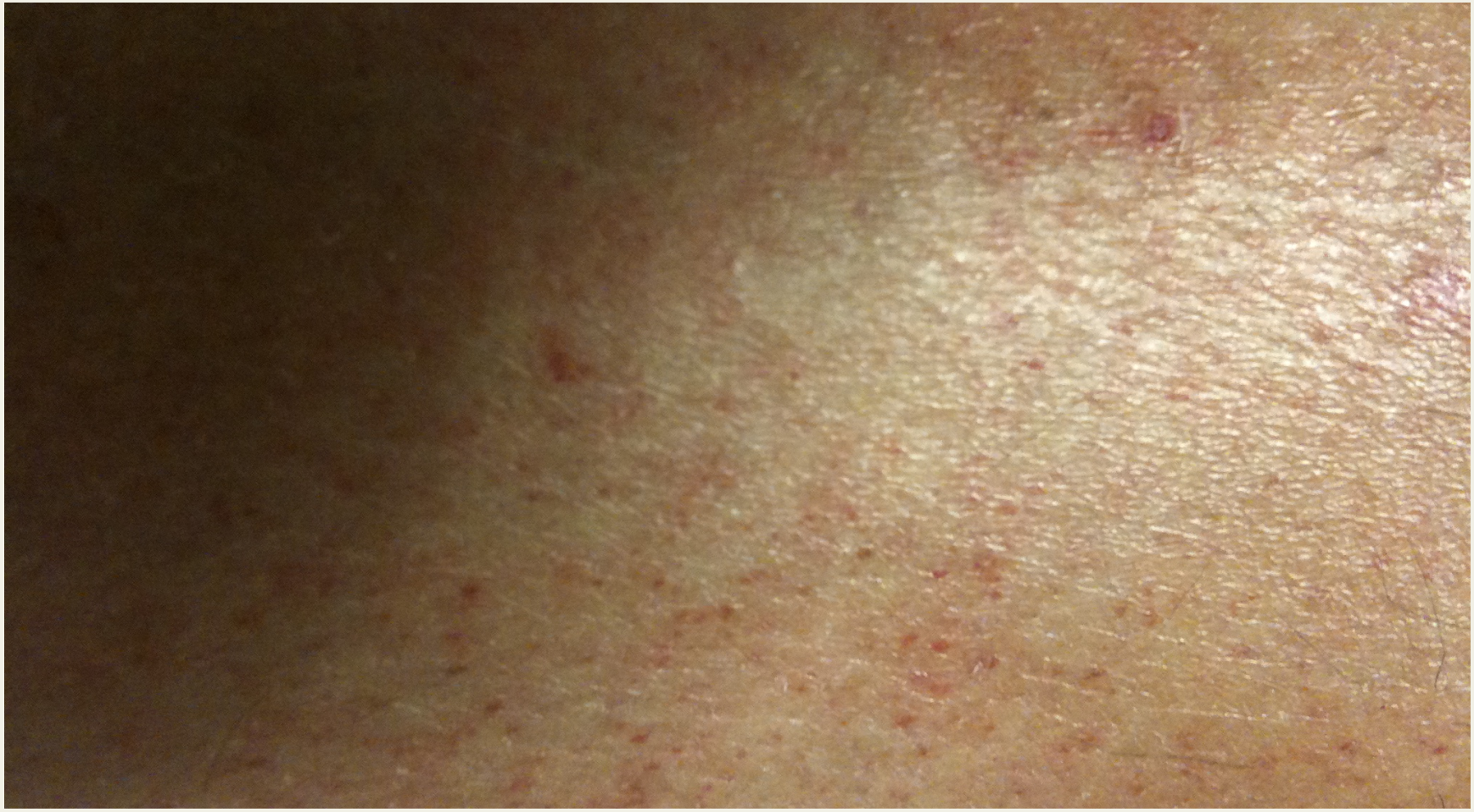
Case Review

- Traveled to India October 12 – 28, 2012
  - Delhi Oct 12 – 17 and 22 – 26, urban area only
  - No malaria prophylaxis, no bednets
- Oct. 28 became ill (headache, mild malaise)
- Oct. 29: worsening headache, chills, rigors, abdominal discomfort. No nausea/vomiting
  - Smear negative for malaria
  - Platelet count 140K, WBC low

Case Review

- Oct. 30: Aches increasing in shoulders, hips and knees, recurrent chills and rigors
  - Oral temperature 100°F
- Oct. 31: continuing chills and moderate arthralgias, severe malaise/lethargy
  - Platelet count 117K, abnormal LFTs
- Nov. 2: Presented to ER for admission: RUQ pain, chills, no fever
  - Platelet count: 15K
  - Petechial rash
- Nov 2 – 4: hospital stay, platelet transfusion
  - Platelets 25K at discharge
- Convalescence 3 – 4 weeks dominated by fatigue and malaise

Petechial Rash



Dengue History

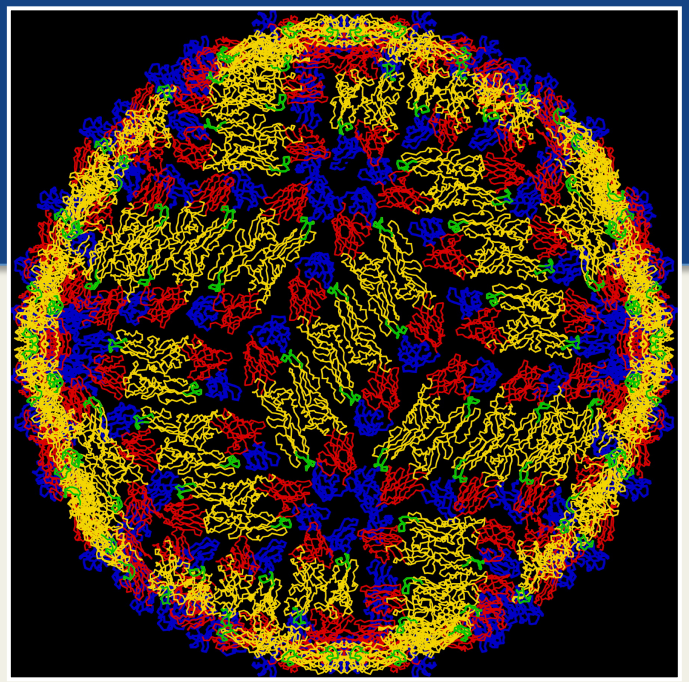
- First clinical description credited to Dr. Benjamin Rush following epidemic of fever in Philadelphia in the summer of 1780
  - "This fever generally came on with rigor, but seldom with a regular chilly fit. . . . The pains which accompanied this fever were exquisitely severe in the head, back, and limbs. The pains in the head were sometimes in the back parts of it, and at other times they occupied only the eyeballs."

Dengue: Background

- Member of the *Flaviviridae* family, genus flavivirus
  - Four DENV serotypes
    - DENV-1
    - DENV-2
    - DENV-3
    - DENV-4
- Positive-sense RNA virus
  - Naked RNA is infectious
- E (envelope) protein is protective

Dengue – member of Flavivirus family

Serocomplexes	Number of Serogroups	Serious Disease Manifestation
Dengue Virus	4	Dengue Fever, DHS/DSS
Japanese Enceph. (JE, WN, KUN, MVE, SLE)	4	Encephalitis
Yellow Fever	1	Encephalitis, Hepatitis
Tick-borne Enceph. (CEEV, FEEV, Langat)	1	Encephalitis



E Protein

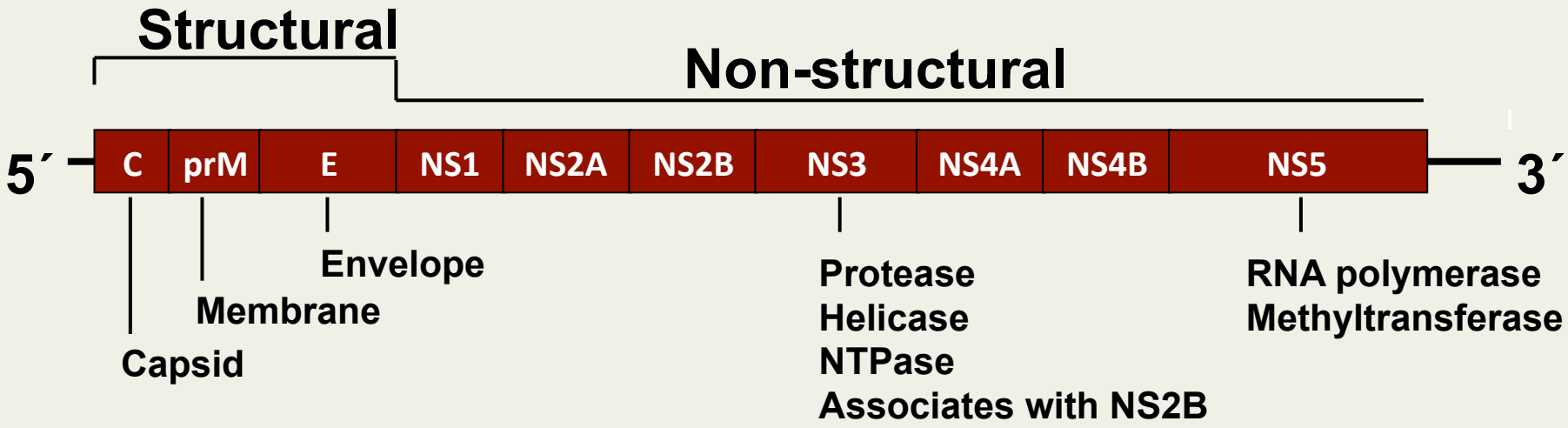
Domain I: red

Domain II: yellow

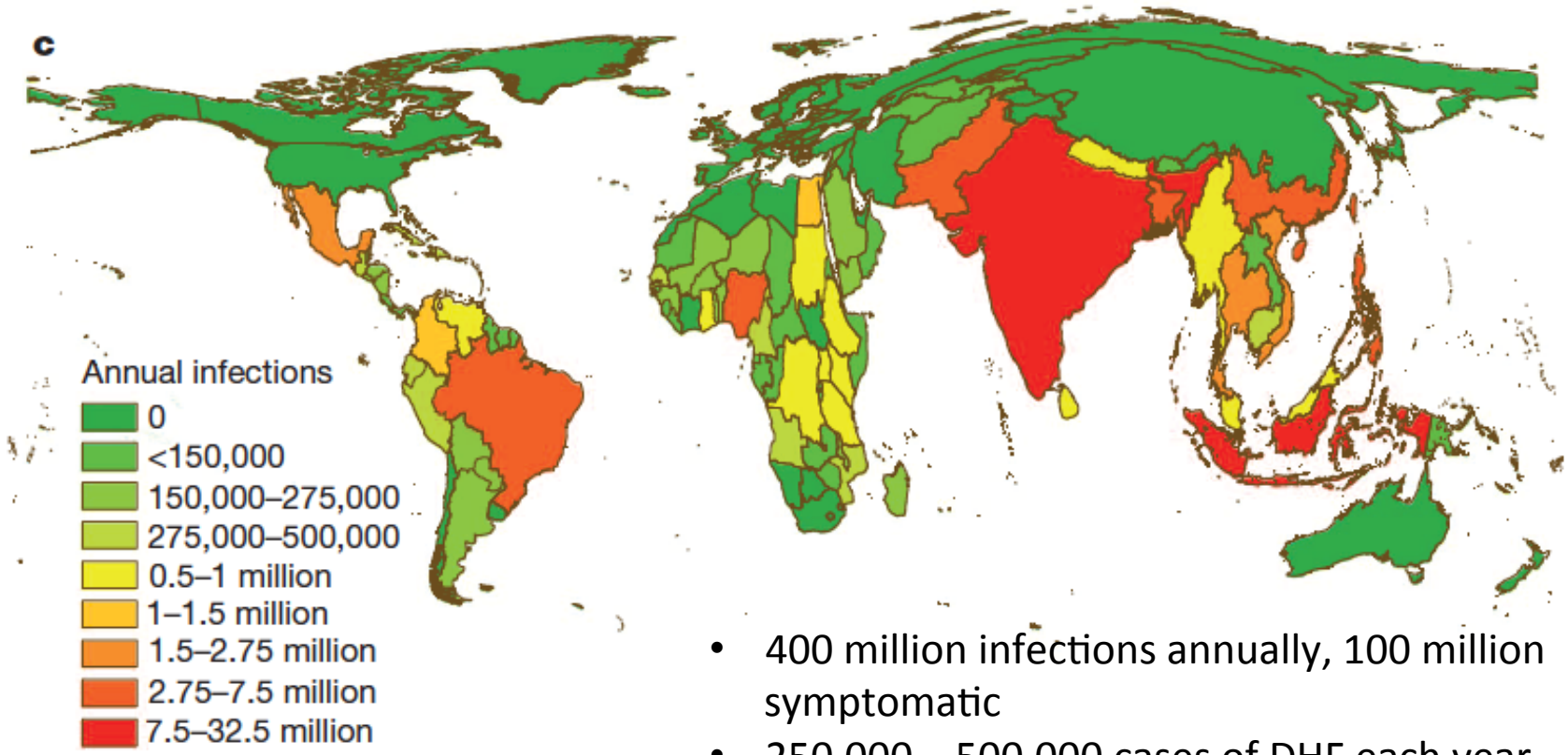
Domain III: blue

Fusion Peptide: green

Source: Kuhn, et al. Cell. 2002 Mar 8;108(5):717-25.



Annual Burden of Dengue



- 400 million infections annually, 100 million symptomatic
- 250,000 – 500,000 cases of DHF each year

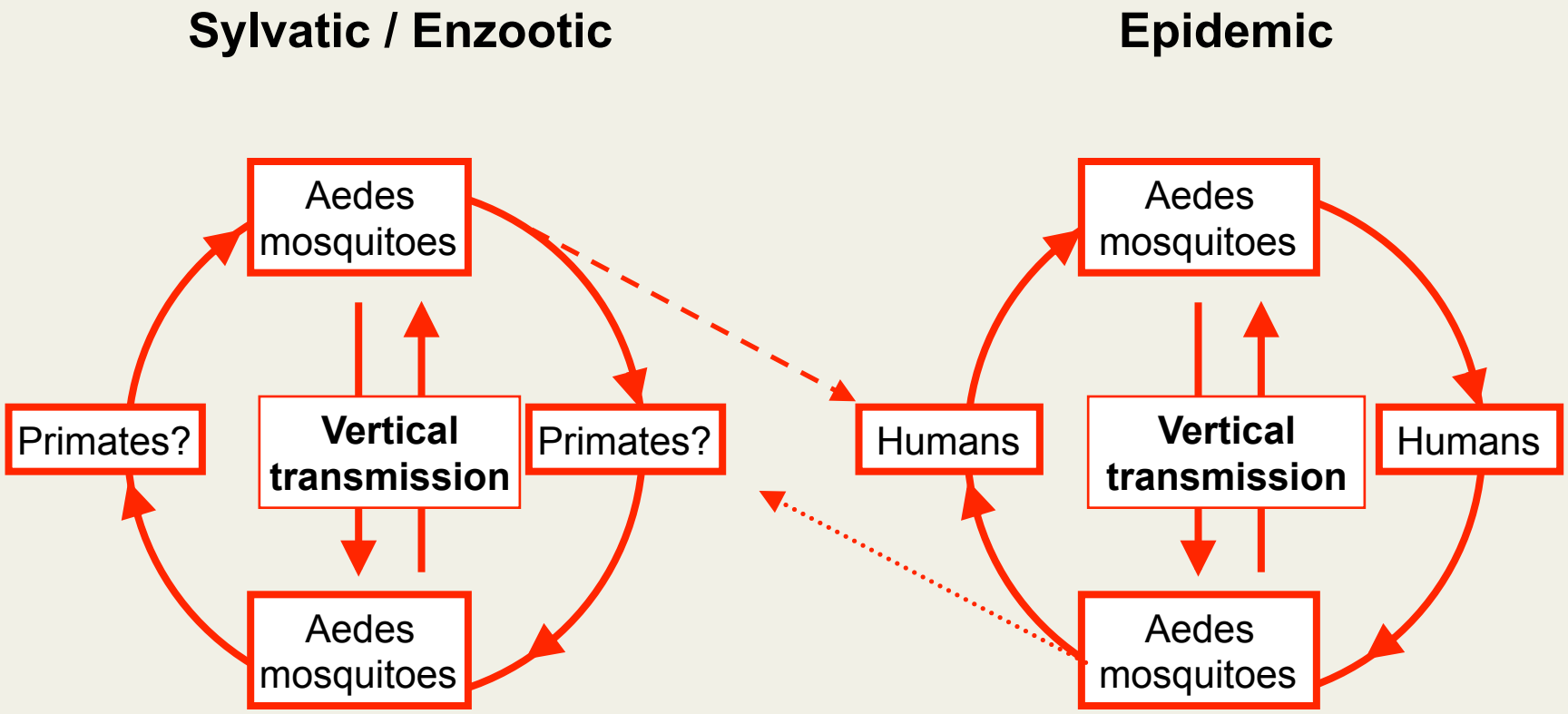
Bhatt et al, Nature 2013

Dengue

- Each serotype provides long-term homotypic immunity but only short-term heterotypic immunity
- **Antibody to E protein protective**
- There is genetic variation within serotypes
- Some genetic variants within each serotype may be more virulent or have greater epidemic potential than other variants

Epidemiology

Dengue Virus - Transmissibility

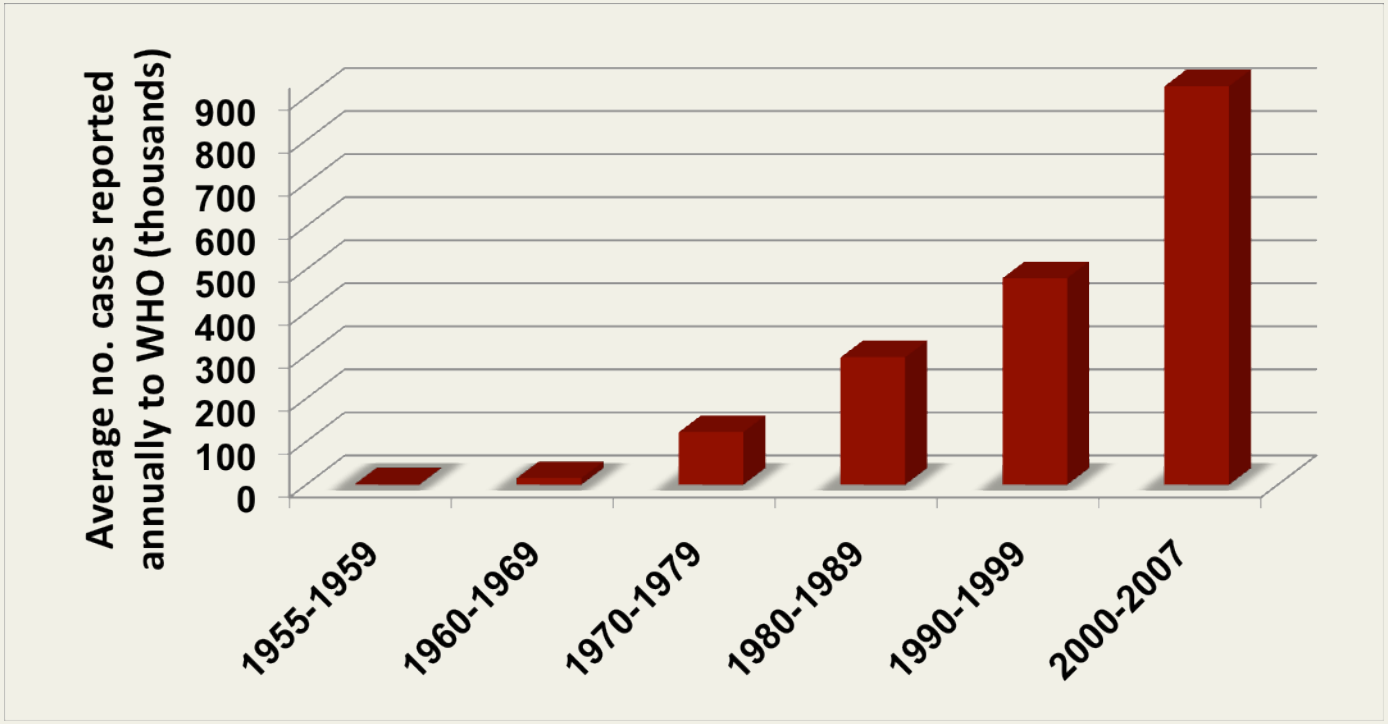


Aedes Aegypti

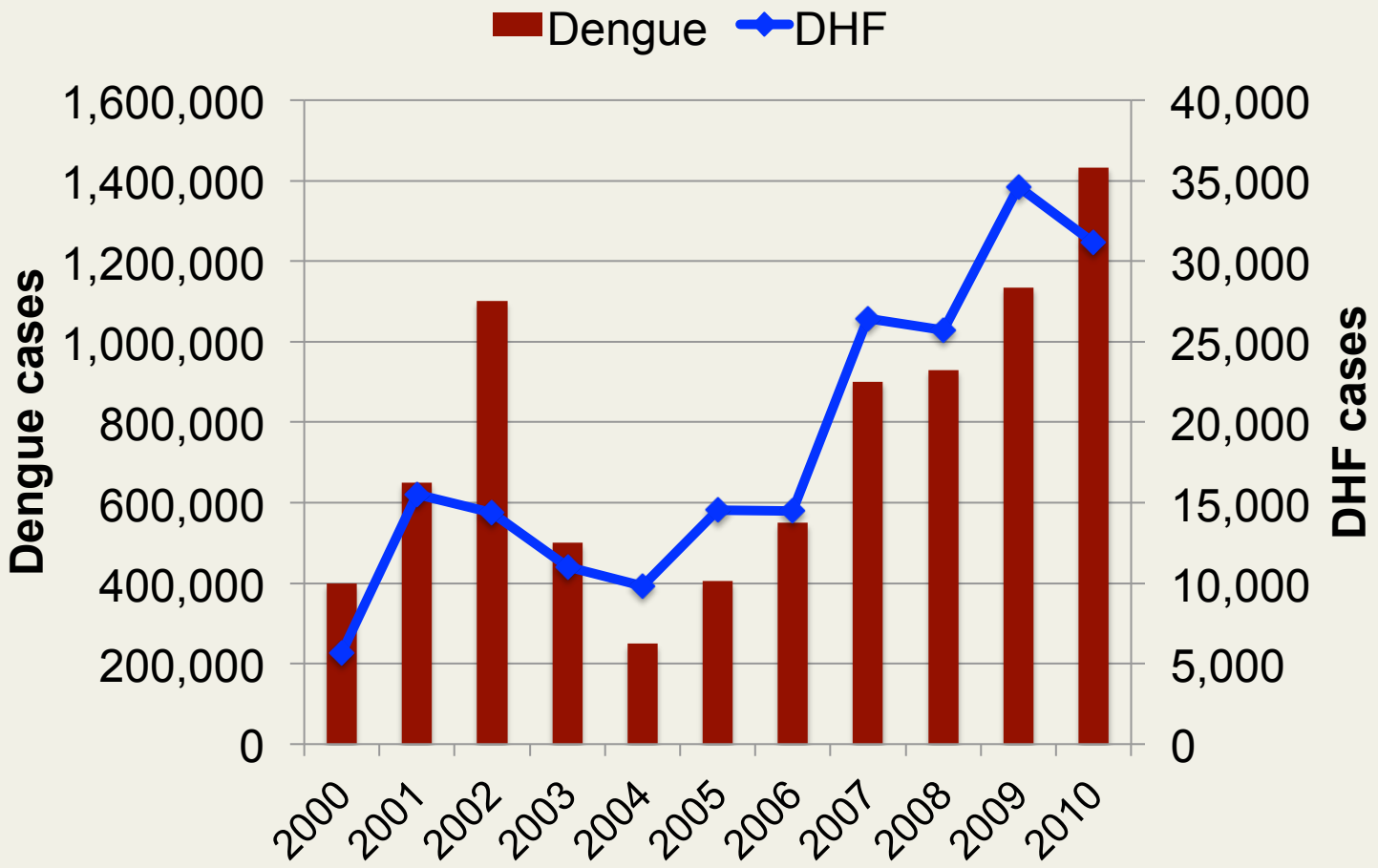
Dengue - Global Resurgence

- Decline in vector eradication programs
  - Ineffective mosquito control in most dengue-endemic countries
  - Ultra-low volume (ULV) spraying of insecticide for adult mosquito control
- Uncontrolled urbanization
  - Inadequate water, sewer, & waste management
  - Increased use of non-biodegradable packaging
- Increased world travel by airplane
- Hyperendemicity-multiple serotypes present

Reported cases of DHF/DSS & death



A decade of dengue in the Americas



Recent Dengue Outbreaks

- Hawaii - 2001 (DEN-1)
  - 118 Cases
- Brazil - 2002
  - 791,192 cases; 2,617 DHF; 145 deaths
- Mexico -Texas border - 2005
  - 1,251 cases, 223 (17.8%) DHF
- Brazil 2008
  - 734,384 cases; 9,957 DHF; 212 deaths
- Brazil 2010
  - 1,004,392 cases; 16,540 severe; 673 deaths
- India 2012
  - ~35,000 cases and ~ 300 deaths

Dengue Resurgence

COMMENTARY

CLINICIAN'S CORNER

JAMA, January 9/16, 2008 Vol. 299

Dengue and Hemorrhagic Fever

A Potential Threat to Public Health in the United States

David M. Morens, MD
Anthony S. Fauci, MD

tries, most notably in Thailand, has greatly reduced case-fatality rates.[11] The economic and social effects of dengue are also enormous because the disease tends to occur in ex-

Centers for Disease Control and Prevention

MMWR Morbidity and Mortality Weekly Report

Weekly / Vol. 59 / No. 19 May 21, 2010

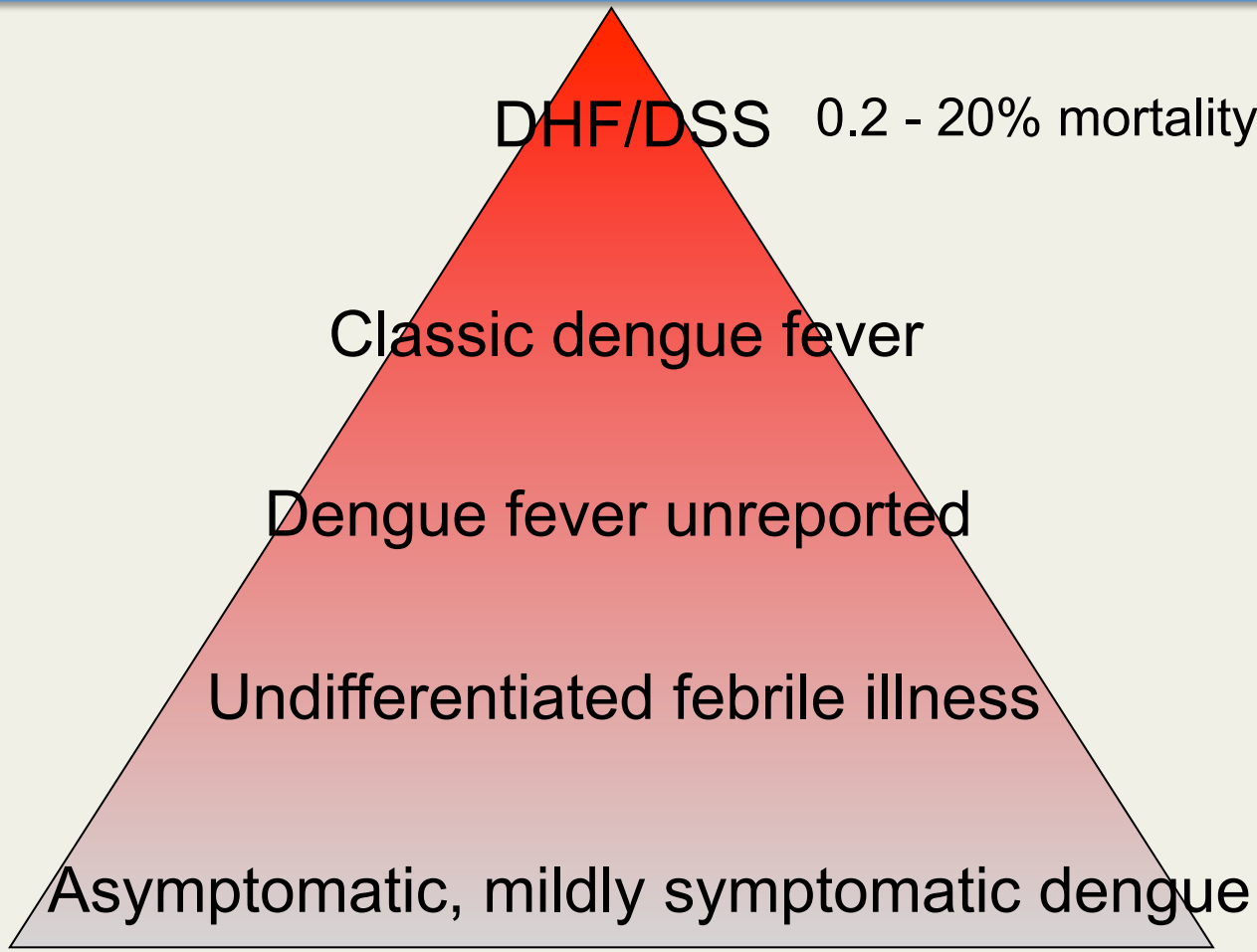
Locally Acquired Dengue — Key West, Florida, 2009–2010

Dengue Illness

Dengue

- DF can range from asymptomatic or mild disease (majority) to incapacitating illness
- Easily confused with other febrile illnesses
- More severe forms of disease - dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS)
- DHF/DSS - Mortality rate can range from 0.2% (treated) to as high as 20% (untreated)

Dengue Spectrum of illness



Dengue - spectrum of illness

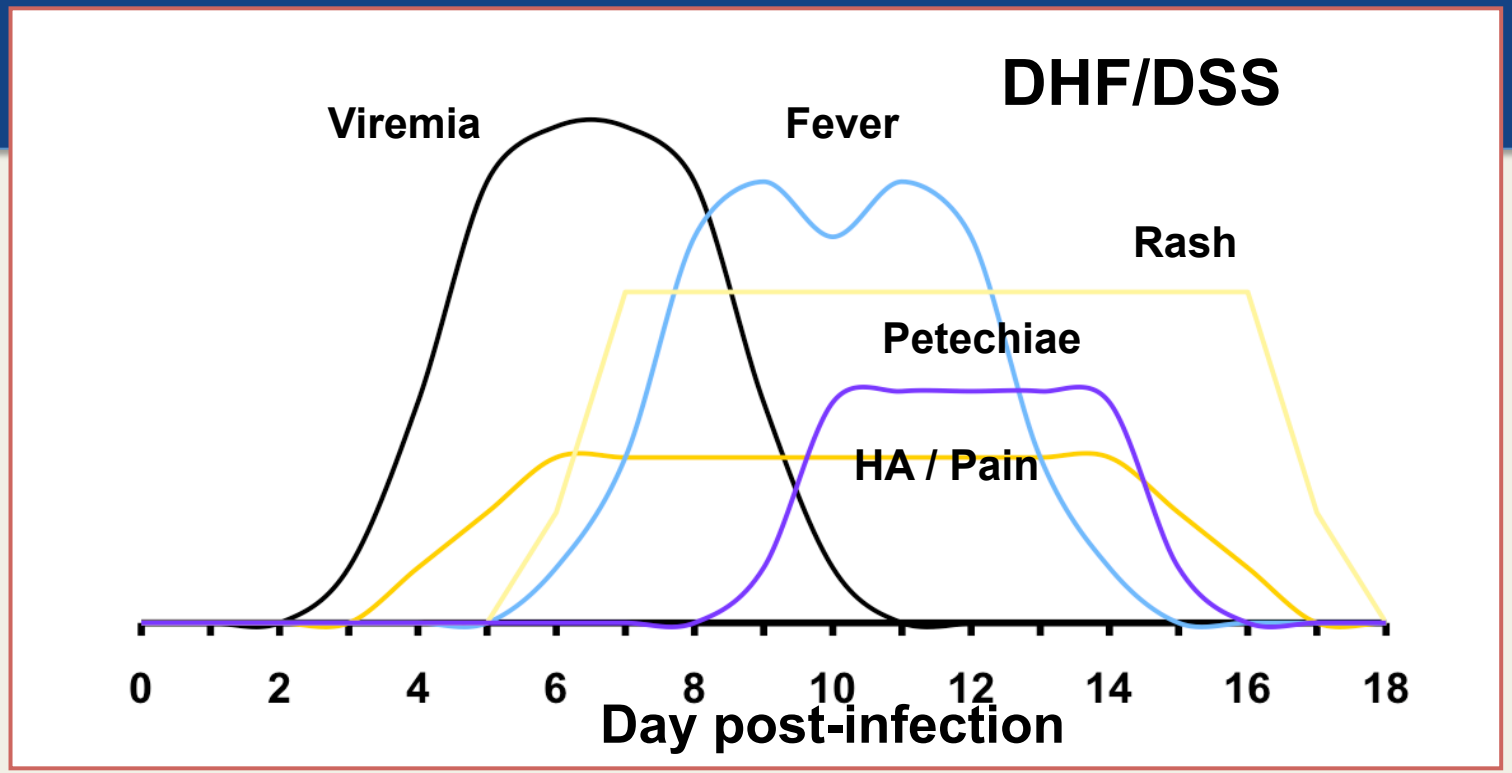
- Classic dengue fever
  - Generally disease of adults
  - Children less symptomatic
- Dengue hemorrhagic fever/shock syndrome
  - Occurs most commonly in 2° infection
  - Generally disease of children < 15 years
  - Occurs in areas hyperendemic for dengue

Dengue Fever: spectrum of illness

- Acute Febrile Illness
- Frontal HA, retro-orbital pain
- Muscle and joint pain (breakbone fever)
- Rash
- Neutropenia

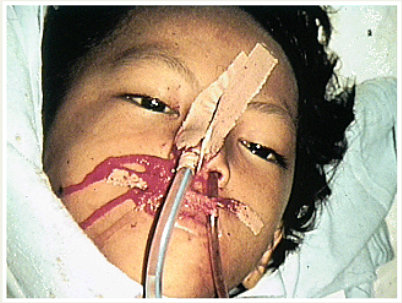
Typical clinical course of DF/DHF/DSS

DF:

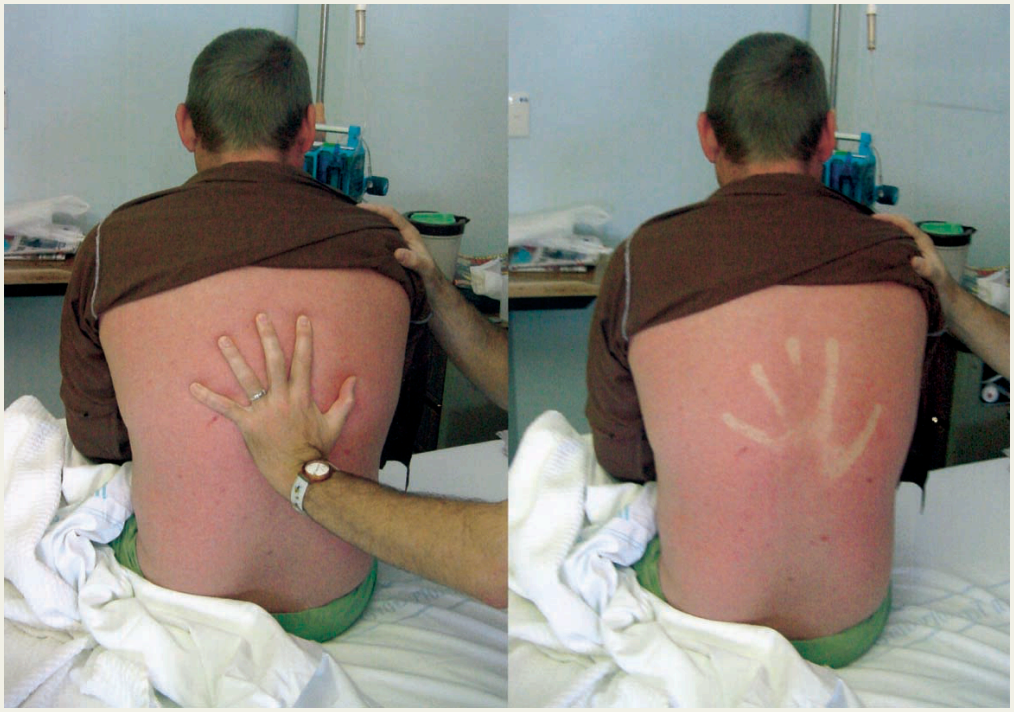


DHF: Hemorrhagic phenomena - petechiae, bruising, bleeding
Hemoconcentration / hypovolemia
Hepatomegaly
Thrombocytopenia / Neutropenia

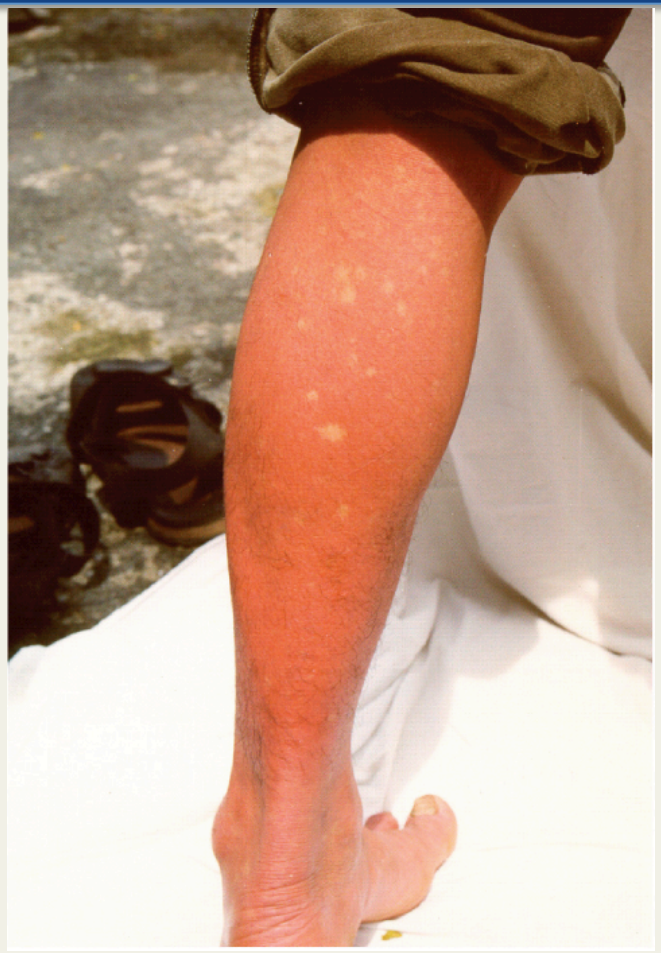
DSS: Rapid weak pulse
Narrowing of the pulse pressure
Circulatory failure: skin cools, cyanosis, shock



Dengue Rash



CID 2004: 38 (15 May)



Moxon, C. Adv Exp Med Biol 2008;609:131-44

DHF/DSS: old WHO criteria

- Dengue hemorrhagic fever: Dengue *plus*
  - Minor or major bleeding (positive tourniquet test)
  - Platelet count < 100,000
  - Evidence of plasma leakage by relative hemoconcentration or by the development of pleural effusion
- Dengue Shock Syndrome (DSS)
  - All the features of DHF *plus*
  - Evidence of cardiovascular compromise due to leakage (pulse pressure ≤ 20mm Hg or hypotension for age with reduced perfusion)
- Criteria under criticism as patient can be extremely ill without meeting the above definitions

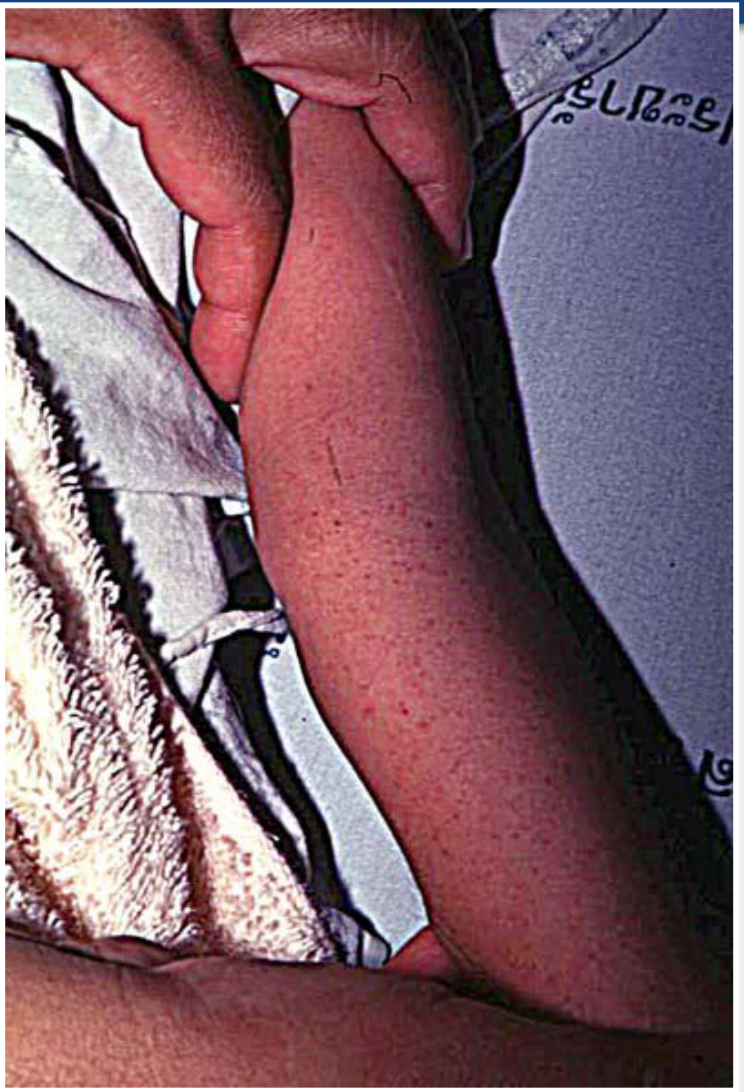
New Dengue Criteria

- Group A: May be sent home
  - Can tolerate oral fluids & do not have warning signs
- Group B: Referred for in-hospital management
  - Patient has warning signs
  - Patient has co-existing condition that may make management more difficult
- Group C: Requires emergency treatment (severe dengue)
  - Severe plasma leakage
  - Severe hemorrhages
  - Severe organ impairment

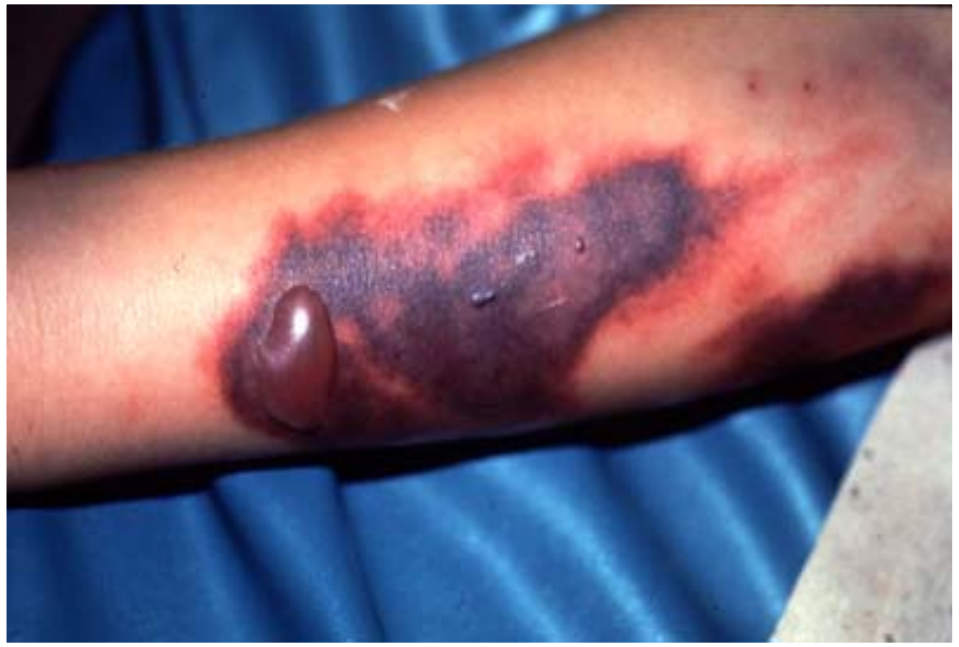
Warning signs

- Clinical
  - Abdominal pain or tenderness
  - Persistent vomiting
  - Clinical fluid accumulation
  - Mucosal bleed
  - Lethargy, restlessness
  - Liver enlargement > 2 cm
- Laboratory
  - Increase in HCT concurrent with rapid decrease in platelet count

DHF-bleeding manifestations



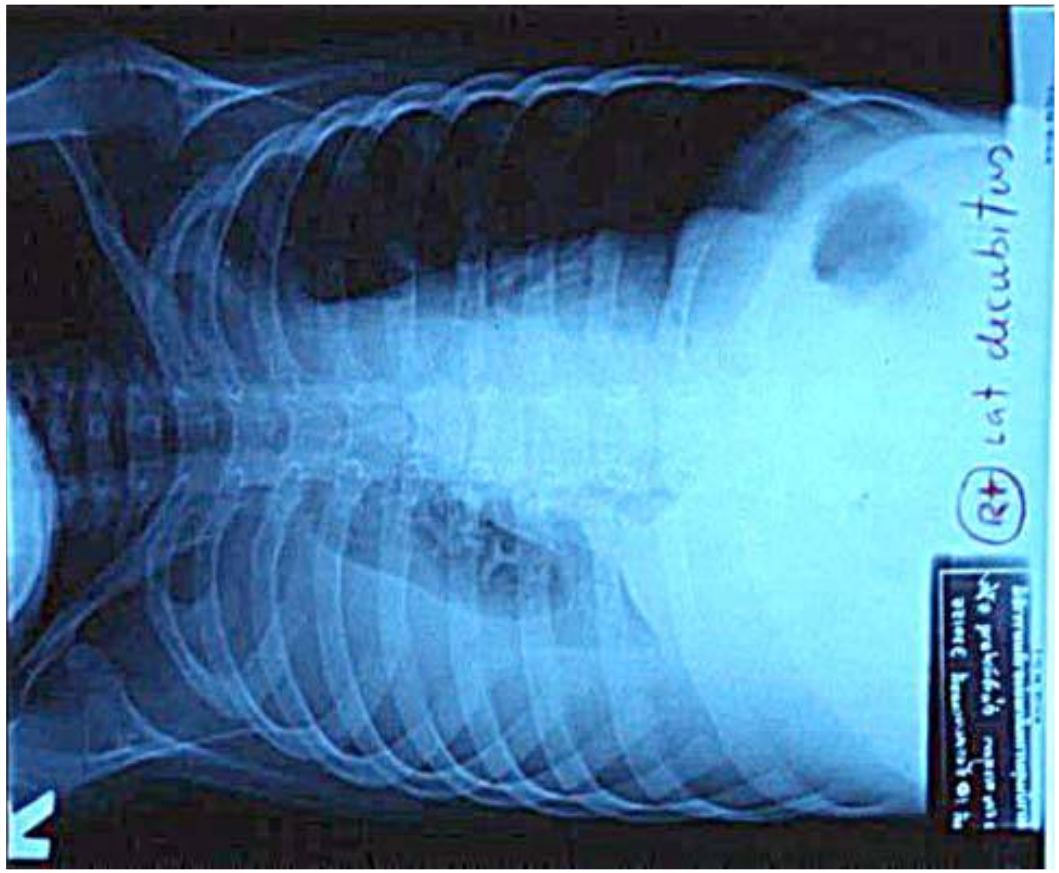
← Petechial rash

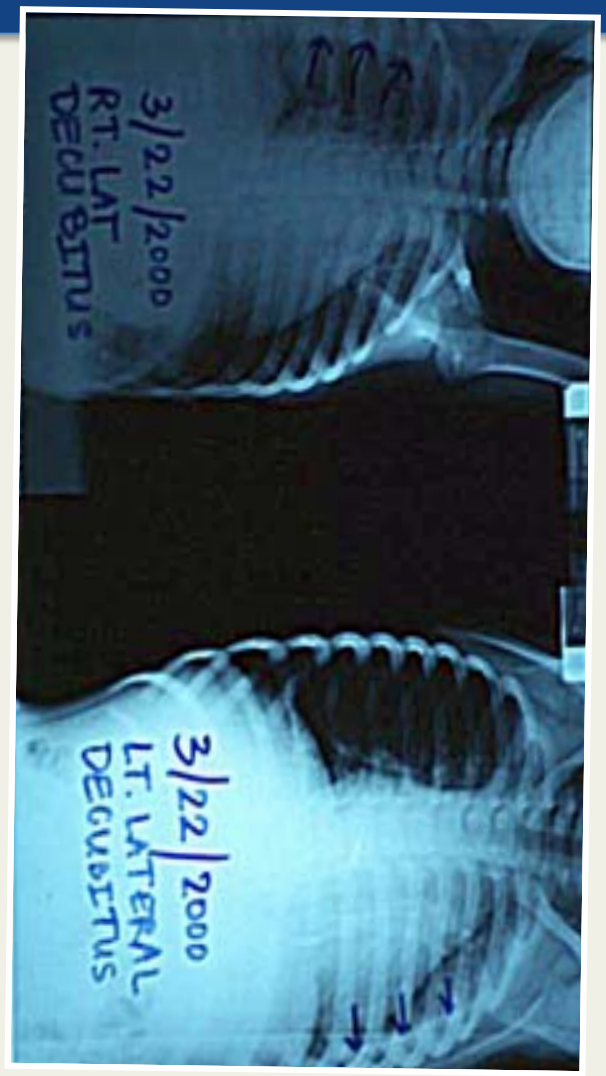


Area of ecchymosis

Courtesy of TDR

DHF/Plasma Leakage





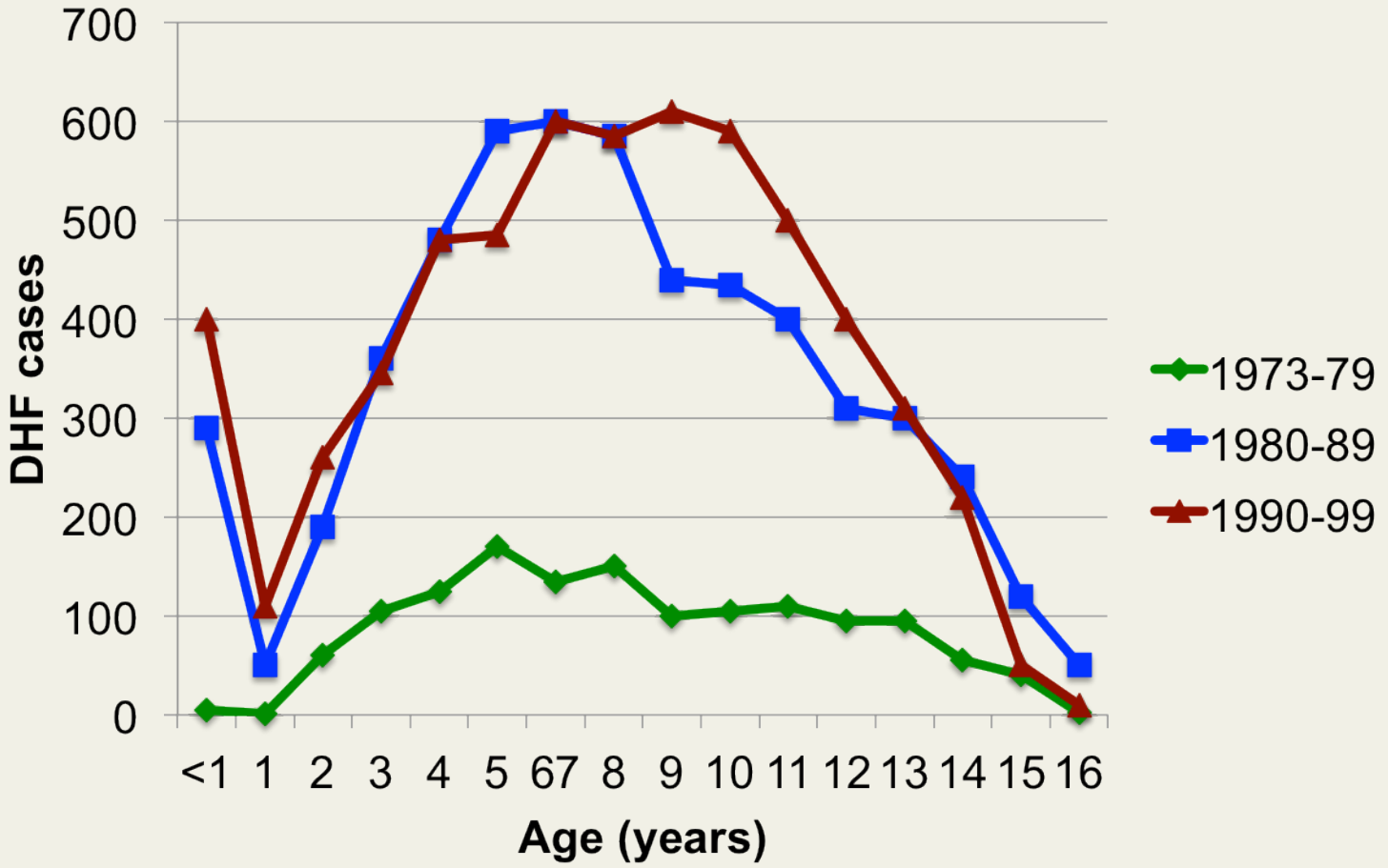
Diagnosis of Dengue

- Diagnosis is made clinically
- Confirmation of diagnosis requires
  - Isolation of virus (culture, PCR, NS1)
  - Serology
    - PRNT
    - ELISA

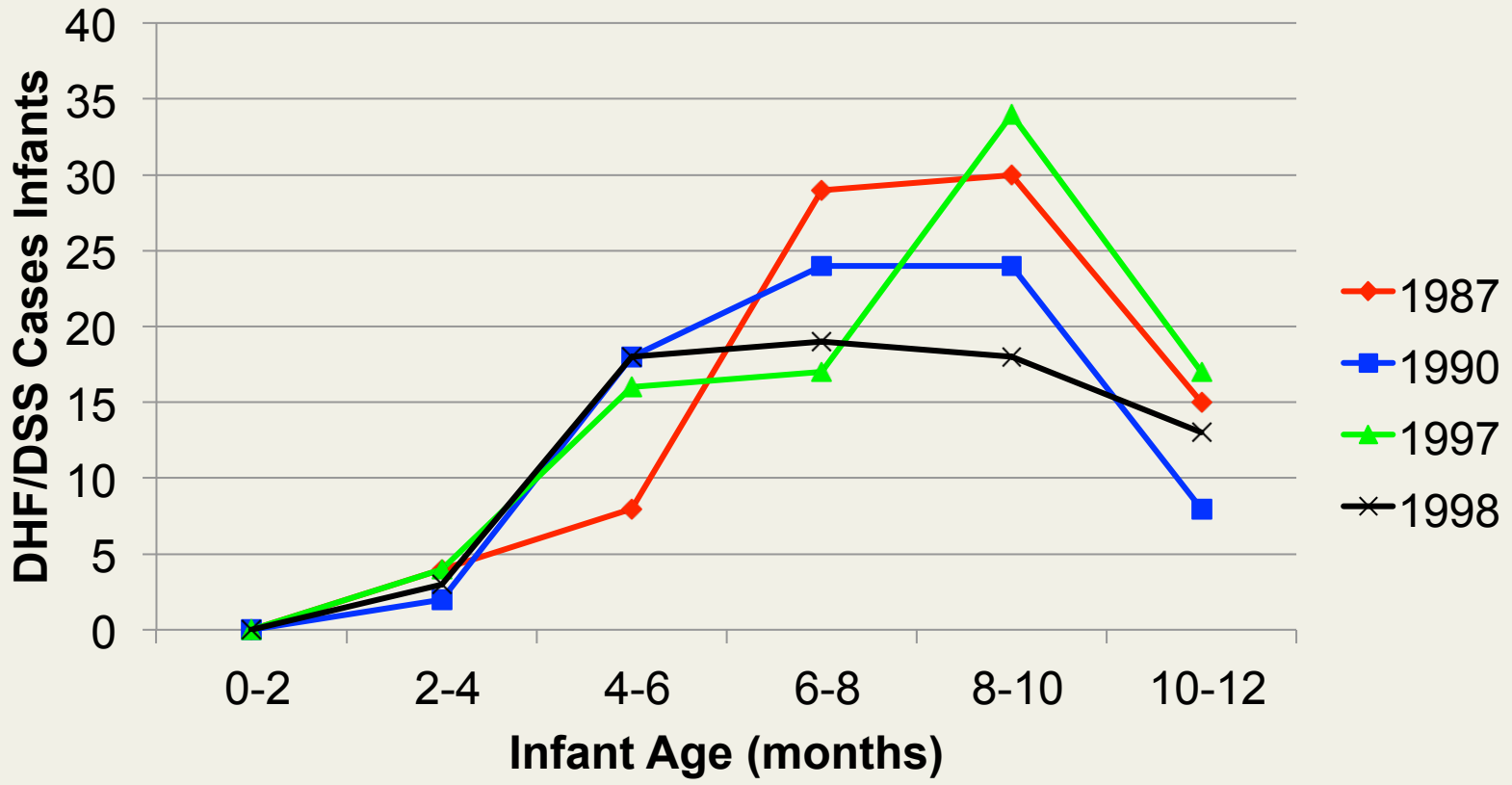
Management of dengue

- Careful management can reduce mortality rate from ~ 20% to < 1%
- Fluid resuscitation is the mainstay of treatment for DHF/DSS
  - If fluid resuscitation not instituted promptly, signs of cardiovascular decompensation progress rapidly
  - With appropriate volume replacement and good supportive care, patients do well
  - Should use isotonic crystalloid solutions
- Avoid nonsteroidal anti-inflammatory agents (ant-platelet effect)

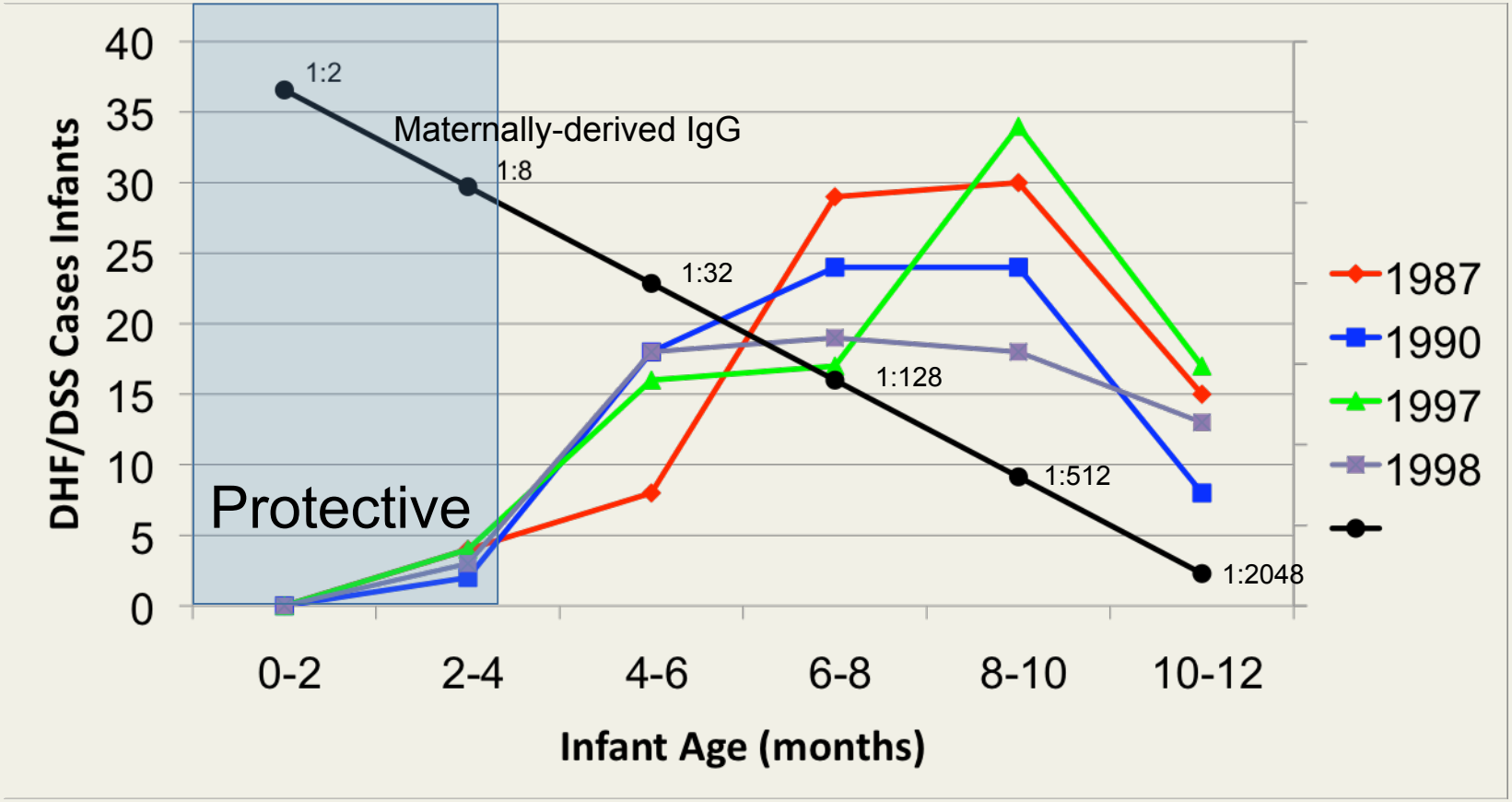
DHF at Bangkok Children's Hospital



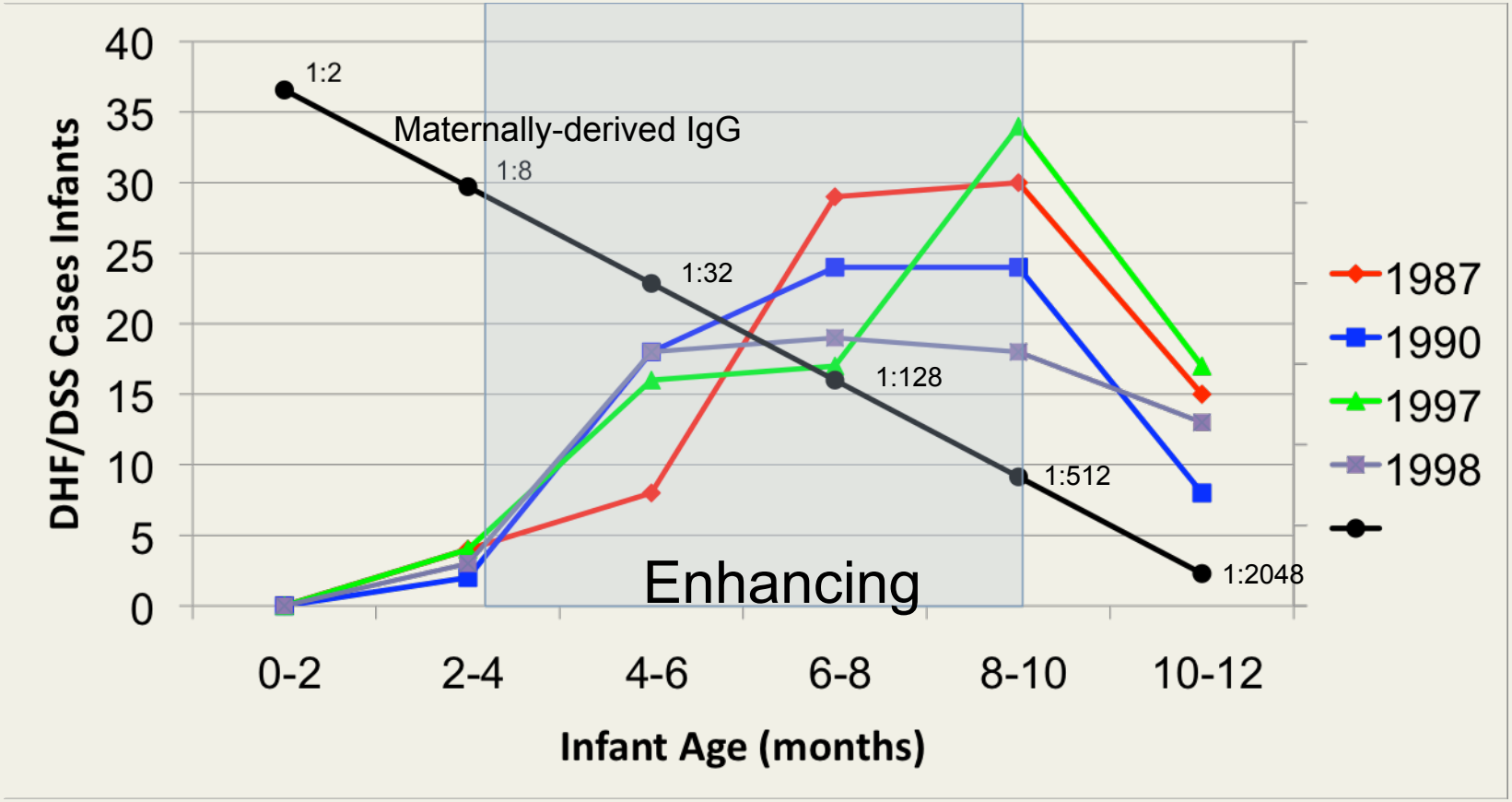
DHF at Bangkok Children's hospital



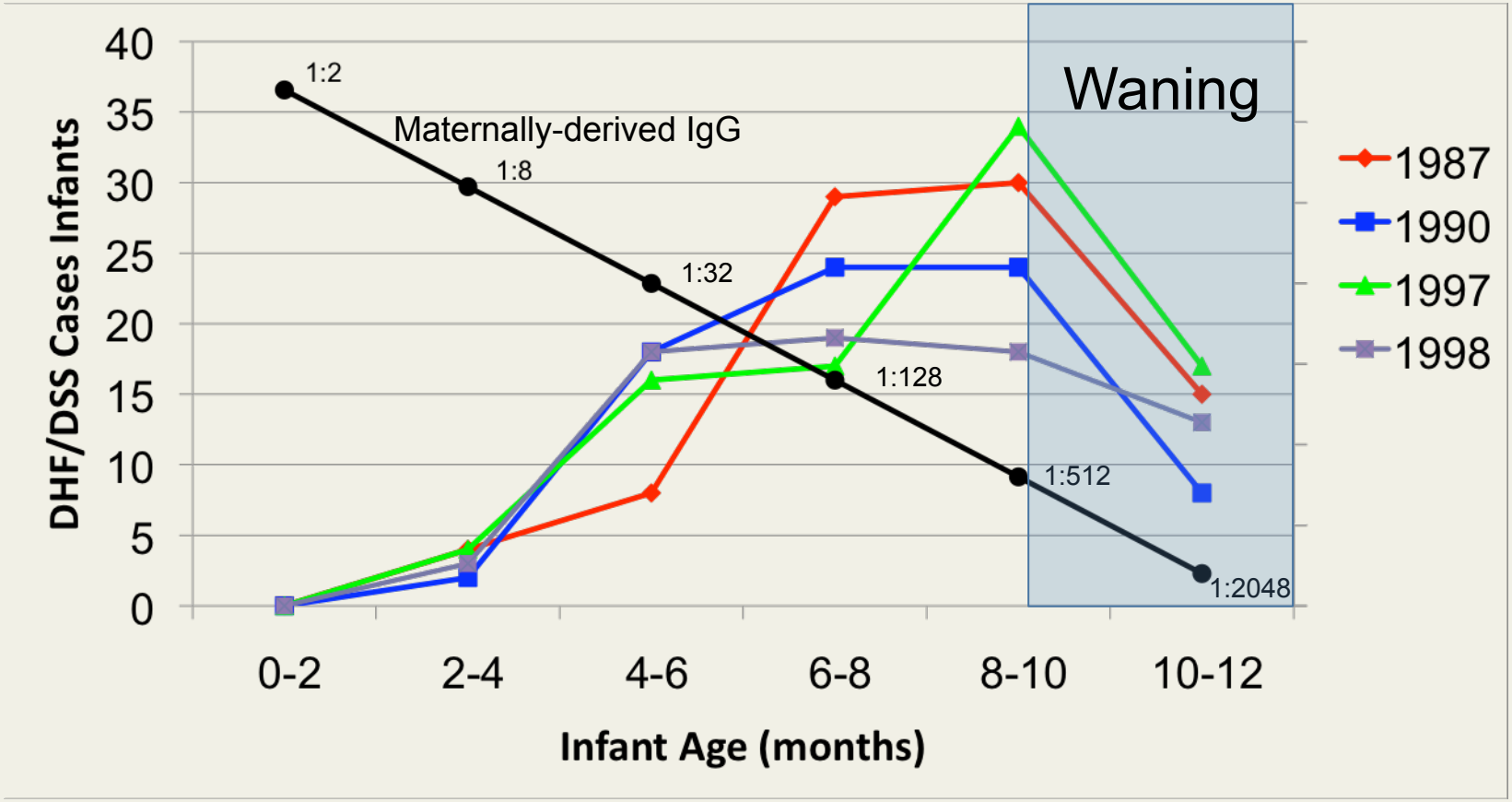
DHF at Bangkok Children's hospital



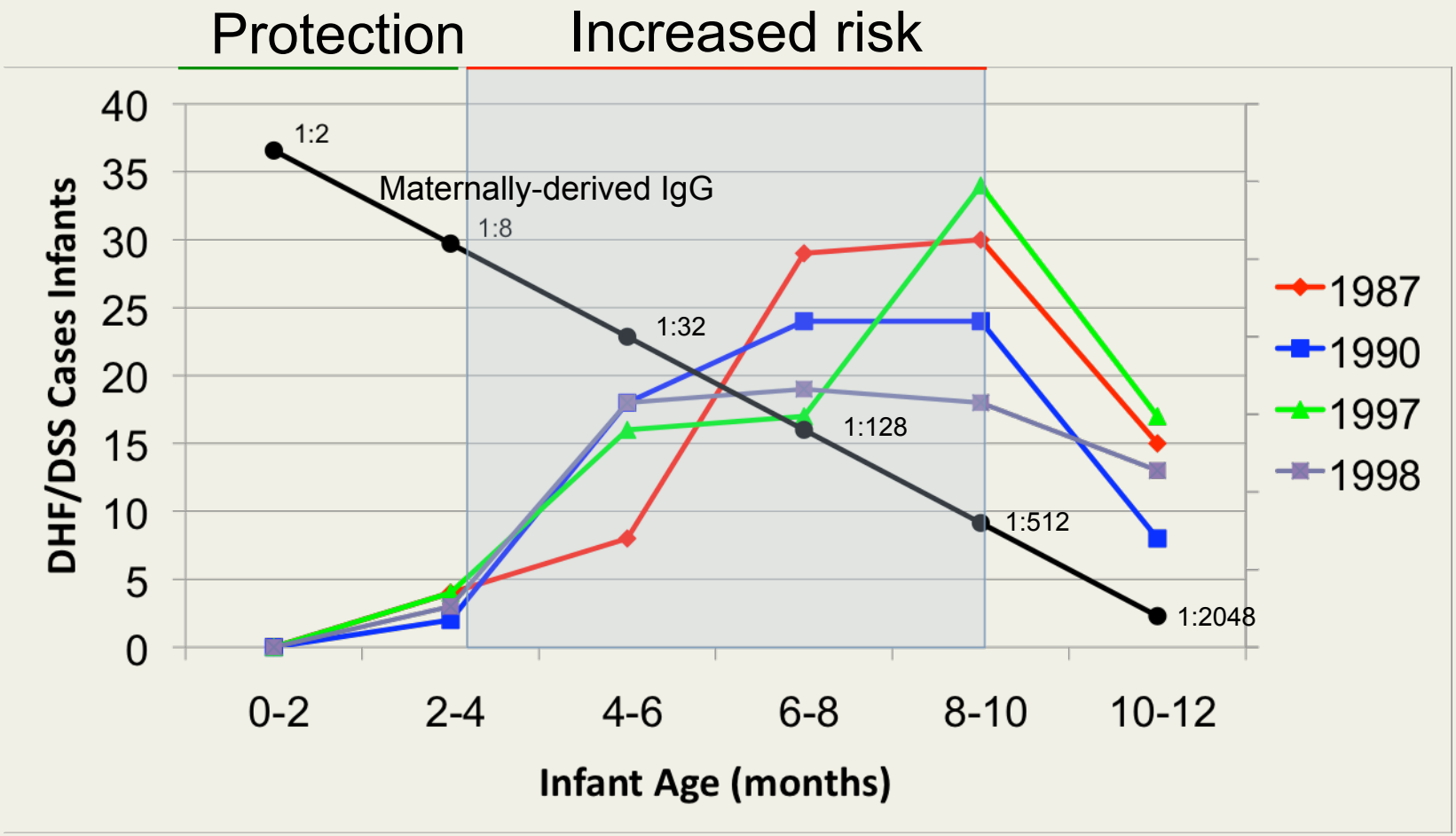
DHF at Bangkok Children's hospital



DHF at Bangkok Children's hospital



DHF at Bangkok Children's hospital

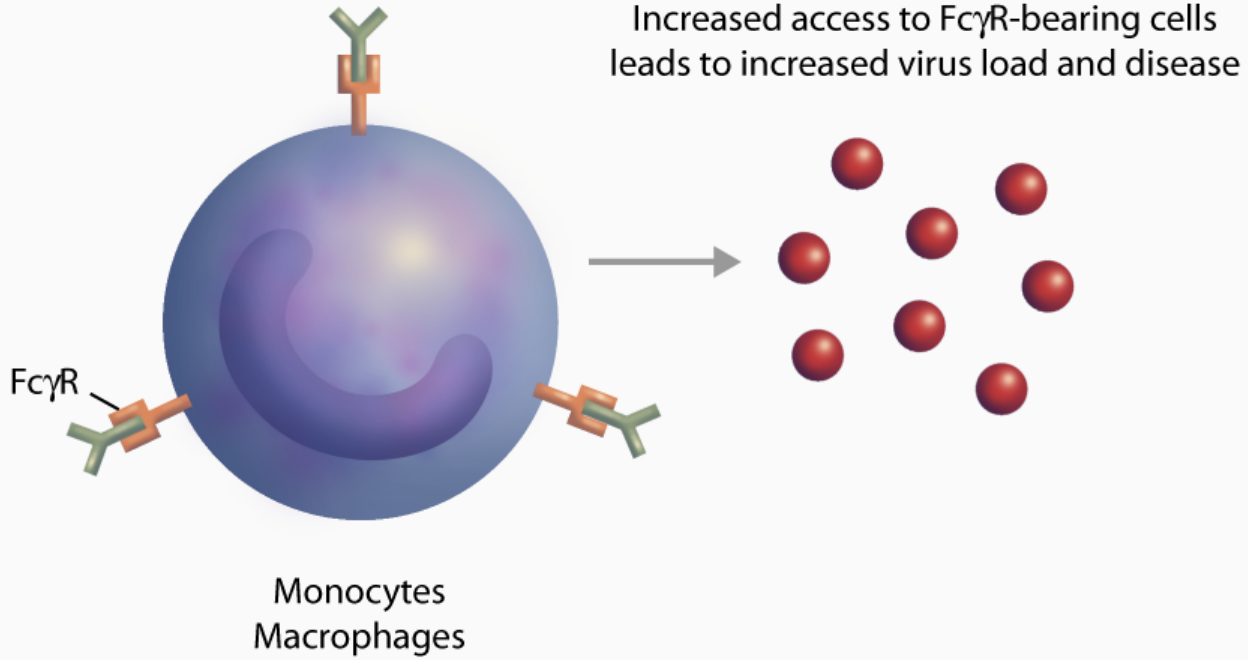


DHF/DSS: Risk Factors

- Secondary infections
  - RR=15 for DHF, RR=50-100 for DSS
- Enhancing antibody
- Age restricted mostly to pediatric group
- Circulation of multiple virus serotypes

Antibody dependent enhancement

- ? Predisposes to DHF/DSS
- Pre-existing cross-reactive antibodies assist virus in entering macrophages via Fc-receptor
- Majority of cases are in secondary dengue infections and in neonates as maternal Ab levels decay


Increased access to FcγR-bearing cells
leads to increased virus load and disease
FcγR
Monocytes
Macrophages
JHSPH
W100907

Prevention

Dengue Prevention

- Currently, the only way to prevent dengue is to prevent mosquito bites
  - Long pants, long-sleeved shirts
  - DEET
- Dengue vaccine development ongoing
- Vaccine must be effective against all 4 serotypes of dengue (tetravalent)

What can we expect from dengue vaccine development?

Stephen Whitehead, 13 January 2015
Demystifying Medicine Lecture

NIH National Institute of Allergy and Infectious Diseases
Leading research to understand, treat, and prevent infectious, immunologic, and allergic diseases.

Important considerations for a dengue vaccine

1. **Dengue disease is caused by any of 4 different serotypes - an effective vaccine must protect against all four viruses**

2. **Infection with one serotype is likely to confer life-long immunity to the homologous serotype and the strains within that serotype**

3. **Sequential infection with different serotypes leads to a broadly neutralizing antibody response**

Important considerations for a dengue vaccine

Most of the millions of adults on the streets of Vietnam are dengue immune.

How did they get that way?

Can we safely induce this immunity in children?

⇩

(Vaccination responses change over time)

Important considerations for a dengue vaccine

- In endemic areas, dengue immunity is most likely acquired by sequential infections, the majority of which are asymptomatic
- This leads to polyclonal neutralizing antibody with broad specificity to all 4 DENV serotypes

Sequential doses		Mean neutralizing antibody on indicated day of second dose		
(2 – 7 year Interval)	Vaccine serotype	Day 0	Day 42	% Sero-conversion
1°	DEN 4	20		
	DEN 1	< 10		
	DEN 2	< 10		
	DEN 3	< 10		

Homotypic

Important considerations for a dengue vaccine

- In endemic areas, dengue immunity is most likely acquired by sequential infections, the majority of which are asymptomatic
- This leads to polyclonal neutralizing antibody with broad specificity to all 4 DENV serotypes

Sequential doses		Mean neutralizing antibody on indicated day of second dose			
(2 – 7 year Interval)	Vaccine serotype	Day 0	Day 42	% Sero-conversion	
1°	DEN 4	20	193	88	Homotypic
2°	DEN 1	< 10	264	100	Homotypic
	DEN 2				
	DEN 3				

Important considerations for a dengue vaccine

- In endemic areas, dengue immunity is most likely acquired by sequential infections, the majority of which are asymptomatic
- This leads to polyclonal neutralizing antibody with broad specificity to all 4 DENV serotypes

Sequential doses		Mean neutralizing antibody on indicated day of second dose			
(2 – 7 year Interval)	Vaccine serotype	Day 0	Day 42	% Sero-conversion	
1°	DEN 4	20	193	88	Homotypic
2°	DEN 1	< 10	264	100	Homotypic
	DEN 2	< 10	169	75	Heterotypic
	DEN 3	< 10	176	75	Heterotypic

Important considerations for a dengue vaccine

But wait!
Dengue immunity acquired by sequential infection is not without its problems.

Primary infection → Secondary infection (different serotype) → Possibility of enhanced disease

Important considerations for a dengue vaccine

1. **Dengue disease is caused by any of 4 different serotypes - an effective vaccine must protect against all four viruses**

2. **Infection with one serotype is likely to confer life-long immunity to the homologous serotype and the strains within that serotype**

3. **Sequential infection with different serotypes leads to a broadly neutralizing antibody response**

4. **Secondary DENV infection with a different serotype is strongly associated with severe disease**

5. **There is no established correlate of protection against DENV: is it neutralizing antibody? cell-mediated immunity? both?**

6. **No usable animal model for DENV disease**

7. **Previously no human challenge model to test vaccine efficacy**

Why develop a live attenuated DENV vaccine?

- Successful for other flaviviruses: YFV and JEV
- Induces both humoral and cellular immune responses
- Presents antigens and epitopes in their native conformation
- Expected to induce lifelong immunity
- Can be very economical to produce
- Highly immunogenic, requiring only one dose

Dosing of live virus vaccines

Vaccine	Primary doses
Mumps, measles, rubella	1
Japanese encephalitis virus	1
Yellow fever virus	1
Adenovirus	1
Smallpox	1
Zoster vaccine	1
Varicella virus	1 or 2
Influenza virus	1 (2 doses ages 2 – 8)
Polio virus	1 - 3
Rotavirus (pentavalent)	3

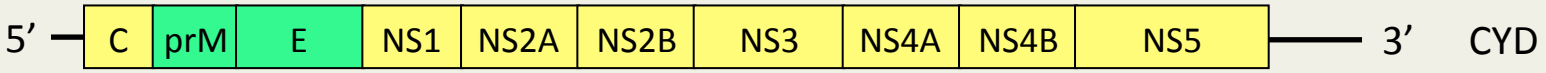
Mucosal administration

Current DENV vaccine "pipeline"

Live attenuated vaccines

Sanofi Pasteur / Acambis

Recombinant YF-17D based chimeric LAV (CYD)



Recently completed efficacy trials in Asia and the Americas

Takeda / Inviragen / CDC

Recombinant DENV-2 (PDK-53) – based chimeric LAV

Phase II, age descending – Colombia, PR, Singapore, Thailand

LID / NIAID

Recombinant DENV based on Δ30 mutation

Current DENV vaccine "pipeline"

Inactivated / subunit vaccines

Merck & Co. / Hawaii Biotech

- Recombinant, truncated E protein. Four components.
- Phase 1 tetravalent (ISCOMATRIX) – Australia. 3 doses. Results pending

GSK / WRAIR

- Purified, inactivated virus. Four components
- Phase I tetravalent (ASO1 and ASO3) – US and Puerto Rico

Tetravalent response in 92 - 100% of naïve subjects after 2 doses

Schmidt, *et al.*, 2013, ASTMH

NMRC

- prM / E DNA preparation. Four components.
- Phase I with DENV-1. Low rate of seroconversion (42%)

3 Sanofi Pasteur Efficacy Trials

2:1 randomization for vaccine or placebo

3 doses: 0, 6, 12 months --> 13 month follow up

Thailand (CYD 23):

N = 3673 children 4 – 11 years old

Overall efficacy = 30% (By serotype: 56, 9, 75, 100%)

Sabchareon, *et al.*, 2012, *The Lancet*

Indonesia, Malaysia, Philippines, Thailand, and Vietnam (CYD 14):

N = 10060 children 2 - 14 years old

Overall efficacy = 57% (By serotype: 50, 35, 78, 67%)

(67% reduction in hospitalization)

Capeding, *et al.*, 2014, *The Lancet*

Brazil, Colombia, Mexico, Honduras and Puerto Rico (CYD 15):

N = 20875 children 9 - 16 years old

Overall efficacy = 61% (By serotype: 50, 42, 74, 78%)

(80% reduction in hospitalization)

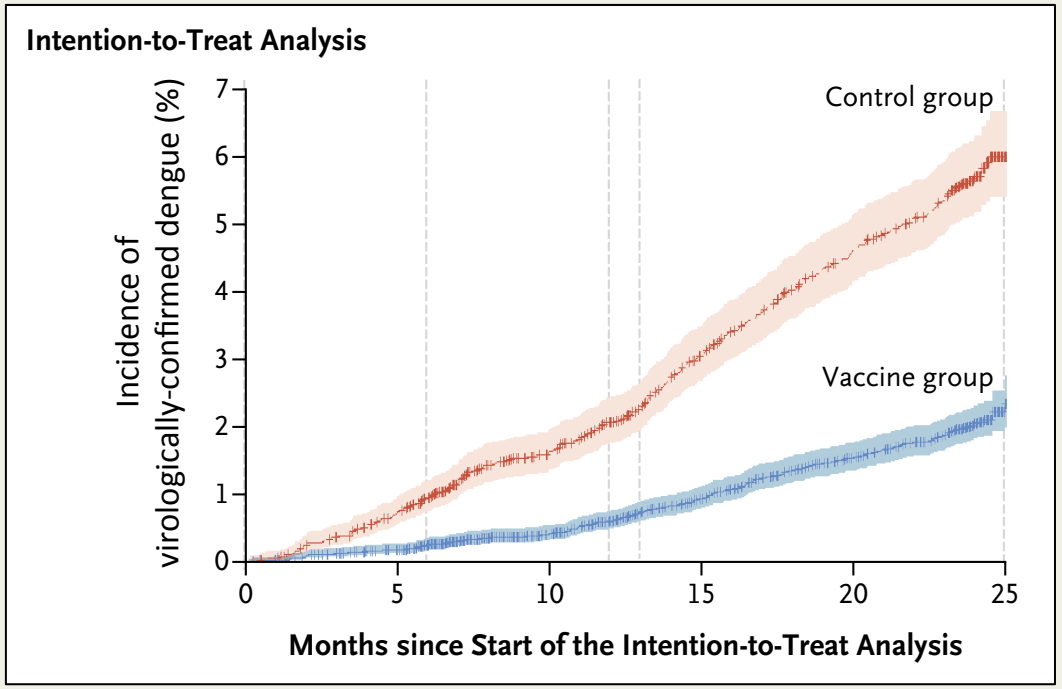
Villar, *et al.*, 2014, NEJM

Sanofi Pasteur Efficacy Trial
Latin America

Brazil, Colombia, Mexico, Honduras and Puerto Rico (CYD 15):

N = 20875 children 9 - 16 years old

Overall efficacy = 61%



Villar, *et al.*, 2014, NEJM

Efficacy begins after the first dose and continues for at least 24 months

Sanofi Pasteur Efficacy Trial
Latin America

Brazil, Colombia, Mexico, Honduras and Puerto Rico (CYD 15):

N = 20875 children 9 - 16 years old

Overall efficacy = 61% (per protocol)

Overall efficacy = 65% (intent-to-treat group – received at least 1 dose)

Analysis from the supplemental material (ITT):

Dengue serostatus at baseline	Vaccine efficacy %
Seropositive	84
Seronegative	43

Villar, *et al.*, 2014, NEJM

Sanofi Pasteur Efficacy Trial
Latin America

Brazil, Colombia, Mexico, Honduras and Puerto Rico (CYD 15):

N = 20875 children 9 - 16 years old

Overall efficacy = 61% (per protocol)

Overall efficacy = 65% (intent-to-treat group – received at least 1 dose)

Analysis from the supplemental material (ITT):

Country	Baseline DENV Seropositivity %	No. of dengue cases by serotype					Vaccine Efficacy %
		DEN1	DEN2	DEN3	DEN4	?	
All	79	109	84	106	83	14	65
Brazil	74	9	0	0	72	0	78
Colombia	92	58	33	67	9	2	68
Honduras	86	6	20	39	0	9	71
Mexico	53	25	30	0	1	2	31
Puerto Rico	56	11	1	0	1	1	58

Villar, *et al.*, 2014, NEJM

✓ **Why does the circulating serotype matter?**

Antibody responses after multiple doses

All studies in flavivirus-naïve subjects:

			Mean titer (GMT) ($PRNT_{50}$)				
Vaccine	N	Dose	DEN1	DEN2	DEN3	DEN4	
CYD Adults US	101	1	9	13	23	643	Dayan. *et al.* 2013 4444 dose
		2					
		3					
CYD 9 – 16 yrs MX, CO, HN, PR	200+	1	8	15	40	120	Villar, L. *et al.* 2013
		2					
		3					

CYD is principally a DEN4 vaccine

Antibody responses after multiple doses

All studies in flavivirus-naïve subjects:

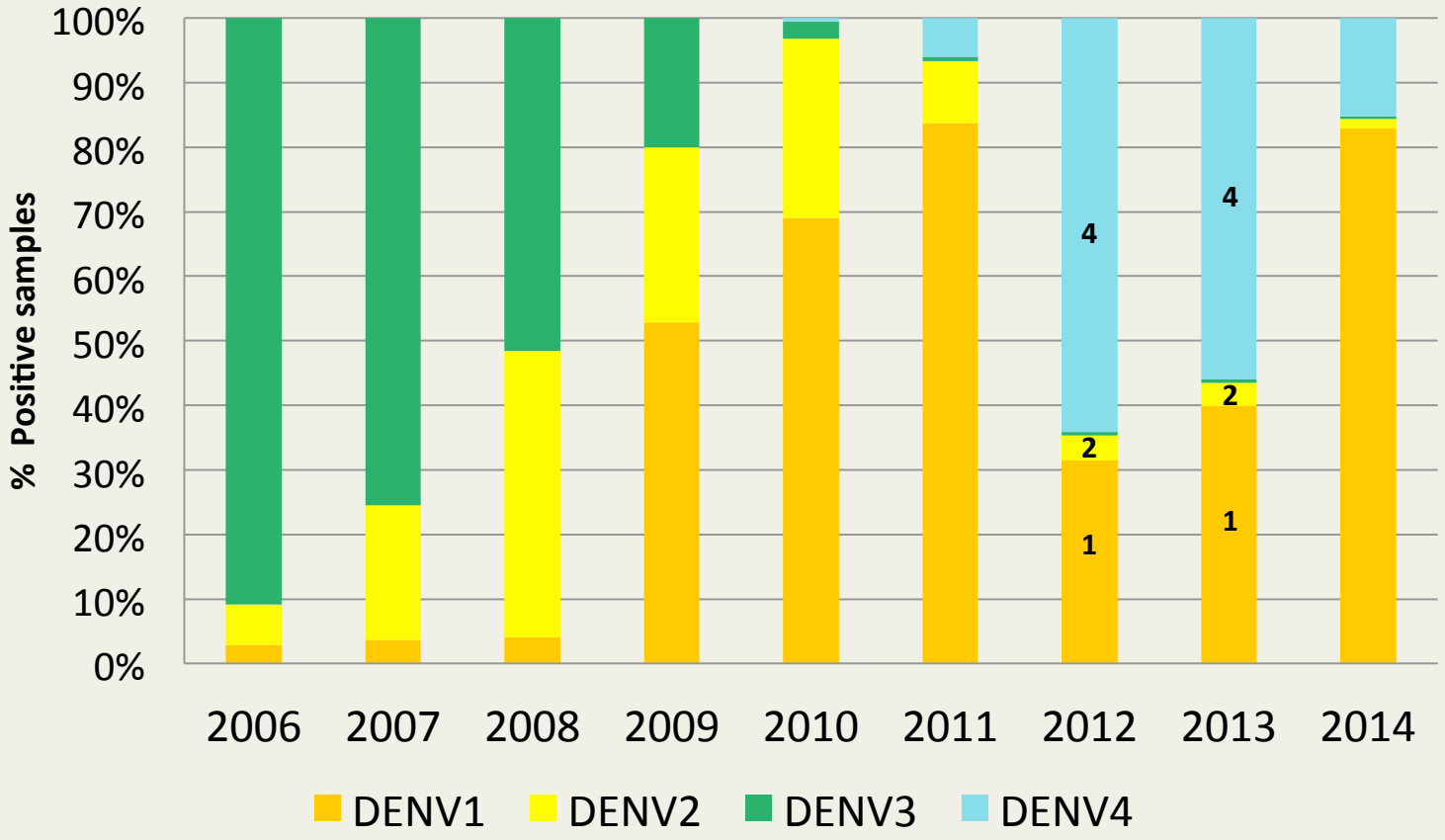
			Mean titer (GMT) ($PRNT_{50}$)				
Vaccine	N	Dose	DEN1	DEN2	DEN3	DEN4	
CYD Adults US	101	1	9	13	23	643	Dayan. *et al.* 2013 4444 dose
		2	19	32	40	164	
		3	24	47	43	134	
CYD 9 – 16 yrs MX, CO, HN, PR	200+	1	8	15	40	120	Villar, L. *et al.* 2013
		2	20	60	100	100	
		3	30	100	120	100	

CYD is principally a DEN4 vaccine

DEN4 response is not boosted at second and third dose

DEN1, DEN2, DEN3 responses increase after subsequent doses

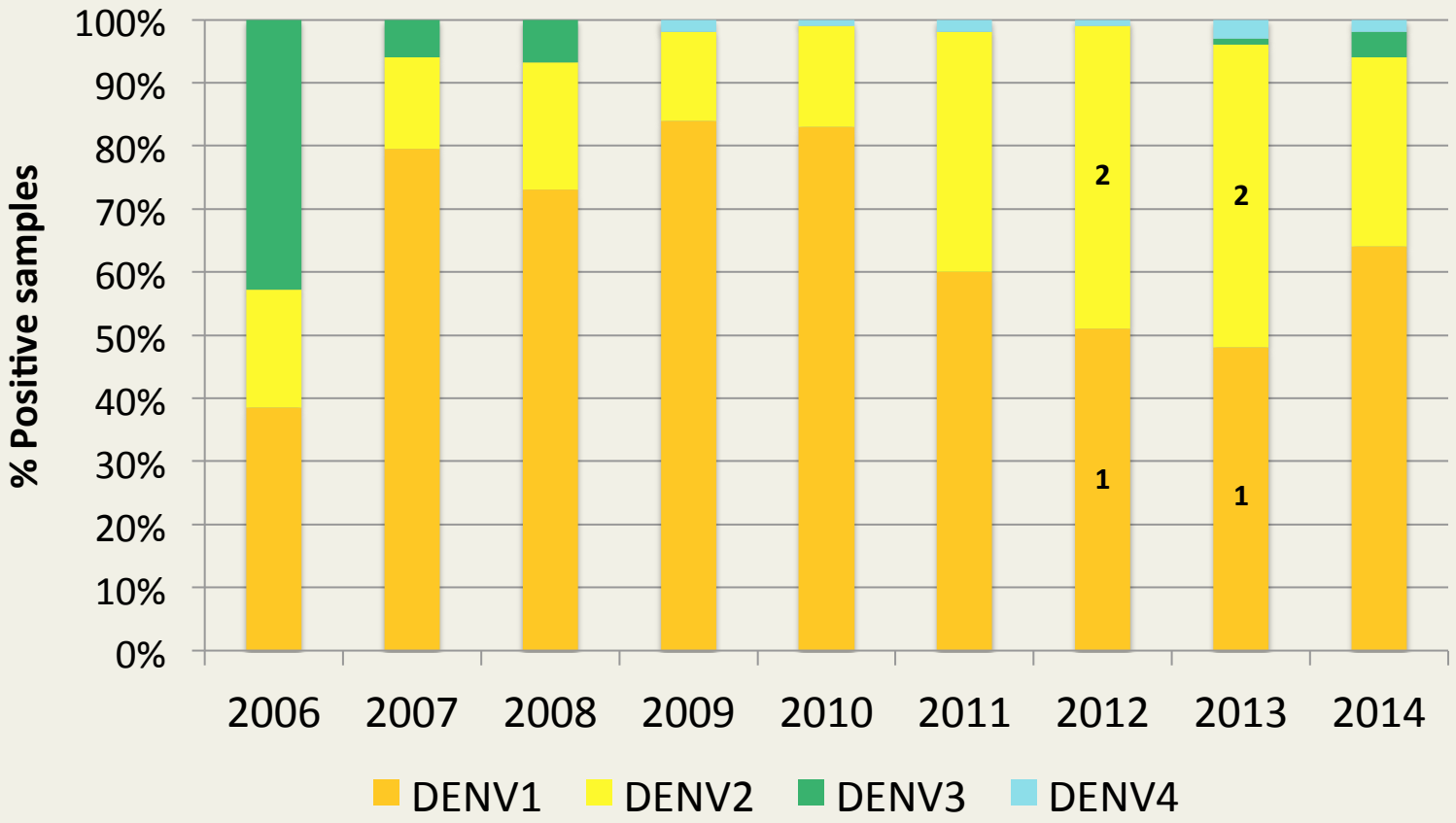
DENV serotype distribution in Brazil



Health Surveillance Secretariat – SVS, Ministry of Health of Brazil, Giovanini Coelho

Efficacy of CYD in Brazil = 78% (74% of subjects were DENV seropositive on Day 0)
Predominant strain circulating in 2012 – 2013 was DEN4

DENV serotype distribution in Mexico



http://www.epidemiologia.salud.gob.mx/dgae/panodengue/historicos_dengue.html

Efficacy of CYD in Mexico = 31% (53% of subjects were DENV seropositive on Day 0)
Predominant strains circulating in 2012 – 2013 were DEN1 & DEN2

Sanofi CYD conclusions

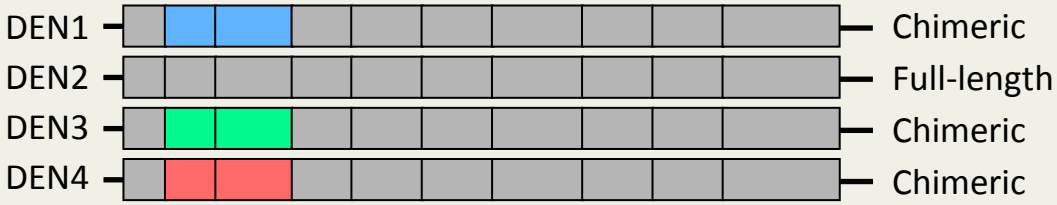
- Principally a monovalent DEN4 vaccine
- DENV priming required for greatest efficacy
- Sequential vaccination strategy
- Low rate of seroconversion in DENV-naïve

  3 doses provides tetravalent response in 78%

  Villar, *et al.* 2011. *Ped. Inf. Dis. J.* Oct 2013.

- CD8+ response directed against YFV non-structural proteins and is unlikely to be cross-reactive with DENV

The Takeda / Inviragen DENVax tetravalent vaccine



Phase I evaluation in dengue-naive adults:

N = 96 adults age 18-45 years old
4:1 randomization for vaccine or placebo
2 dose: 0, 3 months
Low dose: 4, 4, 4, 5
High dose: 4, 5, 5, 5
Subcutaneous or intradermal route (intradermal device)

Osorio, *et al., Lancet*, 24 July 2014.

The Takeda / Inviragen DENVax tetravalent vaccine

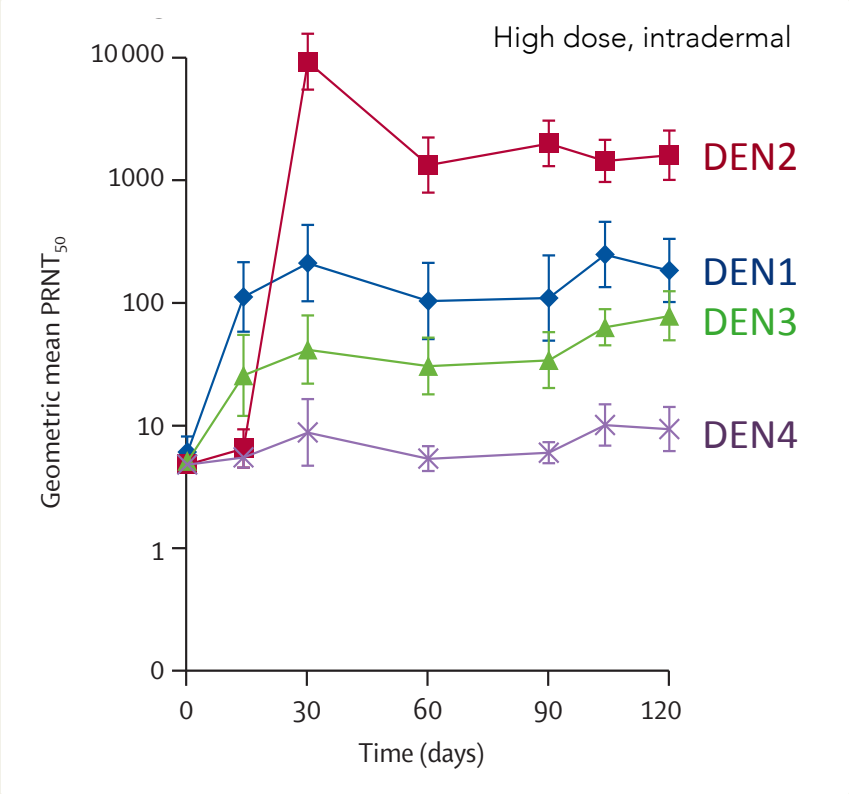
Frequency of tetravalent seroconversion (two dose)

	Low dose	High dose
Subcutaneous	58%	47%
Intradermal	71%	71%

Osorio, *et al., Lancet*, 24 July 2014.

Single-use, disposable Phamajet™ device

The Takeda / Inviragen DENVax tetravalent vaccine



Full-length

Chimeric

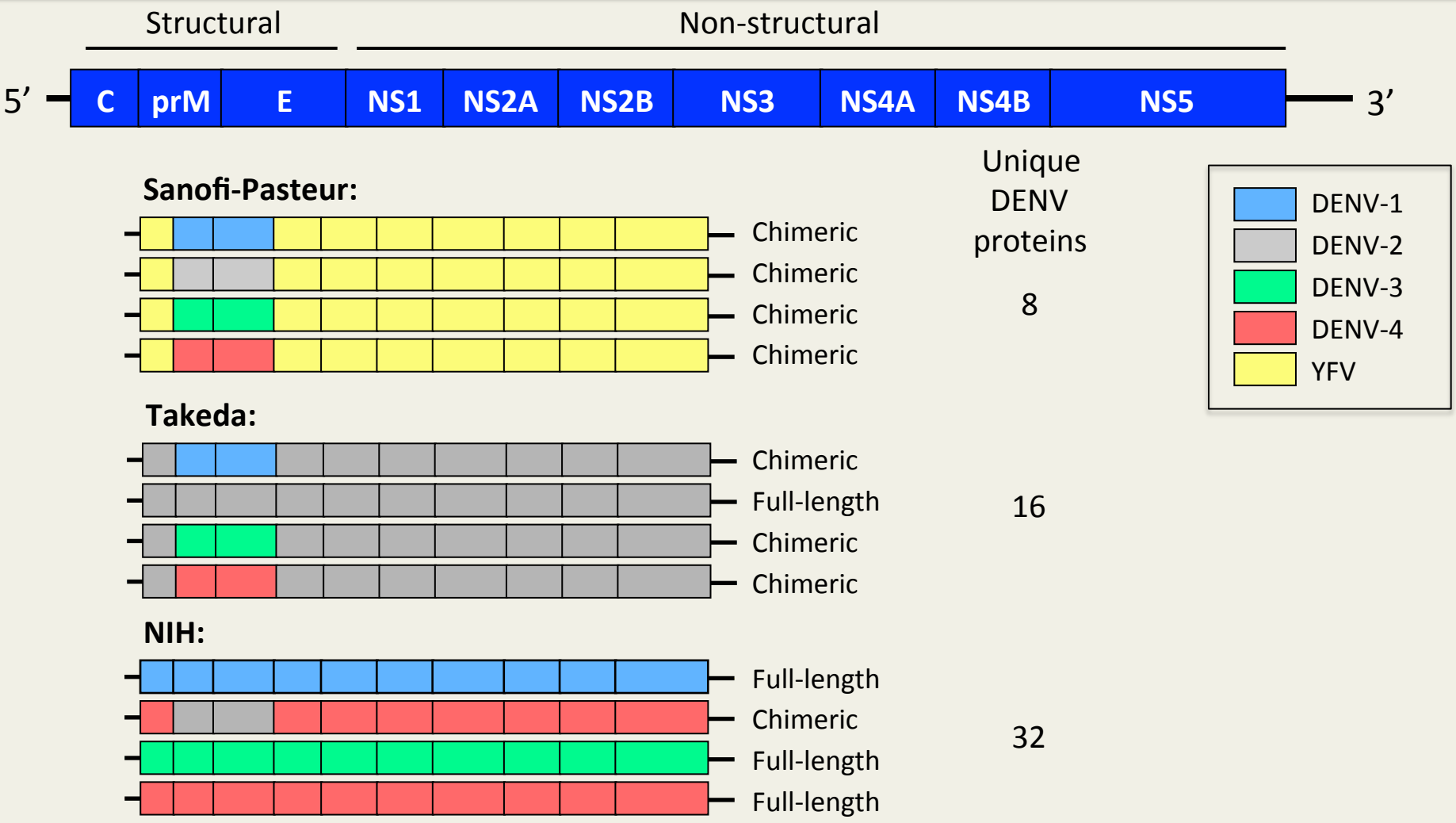
Chimeric

Chimeric

Dose 1		Mean titer (GMT) ($PRNT_{50}$)			
Vaccine	N	DEN1	DEN2	DEN3	DEN4
High dose (-) Adults	24	210	9500	25	9

Osorio, *et al., Lancet*, 24 July 2014.

Recombinant live attenuated DENV vaccine strategies



Dengue Vaccine Development at the LID

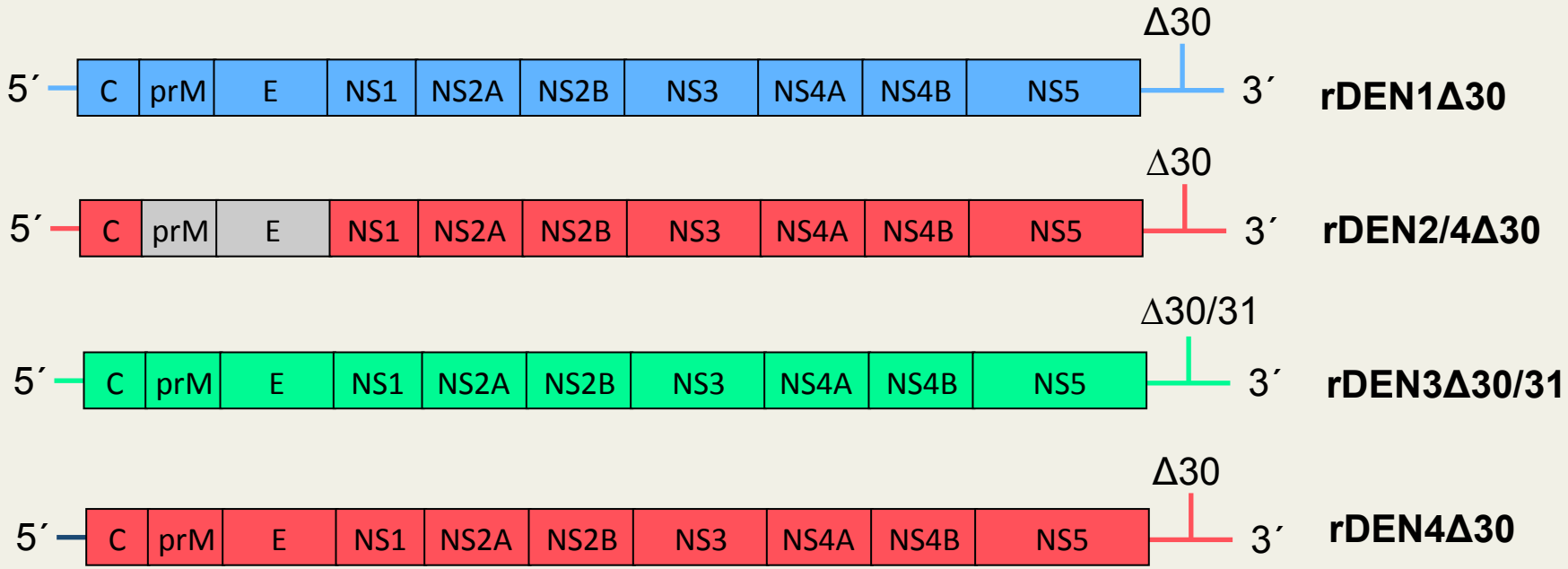
100's of virus constructs

Pre-clinical candidates (monkeys)	Phase I candidates (>700 subjects)	Phase I tetravalent (>175 subjects)	Phase II tetravalent
DEN1Δ30 DEN1/4 DEN1/4Δ30 DEN1/4Δ30(CME) DEN1mutF	DEN1Δ30	DEN1Δ30	**DEN1Δ30**
DEN2Δ30 DEN2Δ30-4995 DEN2/4 DEN2/4Δ30 DEN2/4Δ30(CME) DEN2-3'D4Δ30	DEN2/4Δ30 DEN2Δ30	DEN2/4Δ30	**DEN2/4Δ30**
DEN3Δ30 DEN3/4 DEN3/4Δ30 DEN3/4Δ30(CME) DEN3/4Δ30(Sri Lanka) DEN3Δ86 DEN3Δ30/31 DEN3-3'D4Δ30	DEN3/4Δ30 DEN3Δ30/31 DEN3-3'D4Δ30	DEN3Δ30/31 DEN3-3'D4Δ30	**DEN3Δ30/31**
DEN4Δ30 DEN4Δ30-200,201 DEN4Δ30-436-437 DEN4Δ30-4995	DEN4Δ30 DEN4Δ30-4995 DEN4Δ30-200,201	DEN4Δ30 DEN4Δ30-200,201	**DEN4Δ30**

Safe, highly infectious, balanced immune response

Tetravalent admixture

- All viruses contain wild-type structural proteins
- Contain wild-type NS from 3 of the 4 DENV serotypes
- All contain at least a 30 nt deletion in the 3' UTR



Tetravalent studies in humans

Vaccine	Components:				Potency (log_{10} PFU)
TV-003	DEN1Δ30	DEN2/4Δ30	DEN3Δ30/31	DEN4Δ30	3, 3, 3, 3
TV-005	DEN1Δ30	DEN2/4Δ30	DEN3Δ30/31	DEN4Δ30	3, 4, 3, 3

Healthy adult subjects living in Baltimore, Maryland or Burlington, Vermont
All subjects are flavivirus naïve
Single, subcutaneous administration of tetravalent vaccine
Clinical follow-up every other day through day 16
Serum for viremia collected every other day

Serum Collection for PRNT:

- **Initial: Days 28, 42**
- **Expanded (e): Days 28, 56, 90**

Neutralizing antibody responses

Serum Collection for PRNT
- Initial: Days 28, 42

TV-003: 3, 3, 3, 3
TV-005: 3, 4, 3, 3

		% seroconverted ($PRNT_{50} \geq 10$)				Mean peak titer (GMT) ($PRNT_{50} \geq 10$)			
Vaccine	**N**	**DEN1**	**DEN2**	**DEN3**	**DEN4**	**DEN1**	**DEN2**	**DEN3**	**DEN4**
TV-003	**20**	**100**	**50**	**100**	**100**	**106**	**64**	**42**	**86**
TV-005	**20**	**90**	**60**	**90**	**100**	**52**	**63**	**41**	**76**

Neutralizing antibody responses

Serum Collection for PRNT
- Initial: Days 28, 42
- Expanded (e): Days 28, 56, 90

TV-003: 3, 3, 3, 3
TV-005: 3, 4, 3, 3

		% seroconverted ($PRNT_{50} \geq 10$)				Mean peak titer (GMT) ($PRNT_{50} \geq 10$)			
Vaccine	**N**	**DEN1**	**DEN2**	**DEN3**	**DEN4**	**DEN1**	**DEN2**	**DEN3**	**DEN4**
TV-003	**20**	**100**	**50**	**100**	**100**	**106**	**64**	**42**	**86**
TV-003 e	**38**	**92**	**76**	**97**	**100**	**63**	**40**	**85**	**151**
TV-005	**20**	**90**	**60**	**90**	**100**	**52**	**63**	**41**	**76**

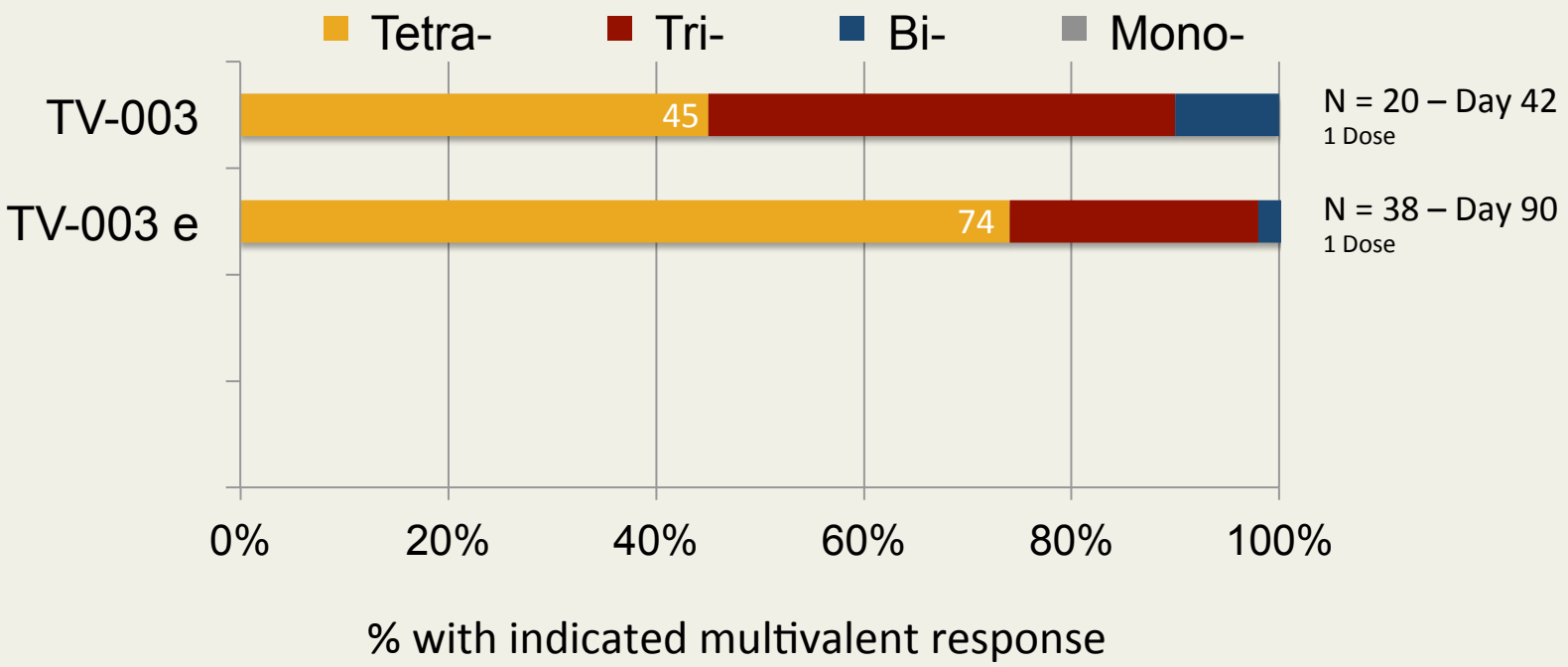
Neutralizing antibody responses

Serum Collection for PRNT
- Initial: Days 28, 42
- Expanded (e): Days 28, 56, 90

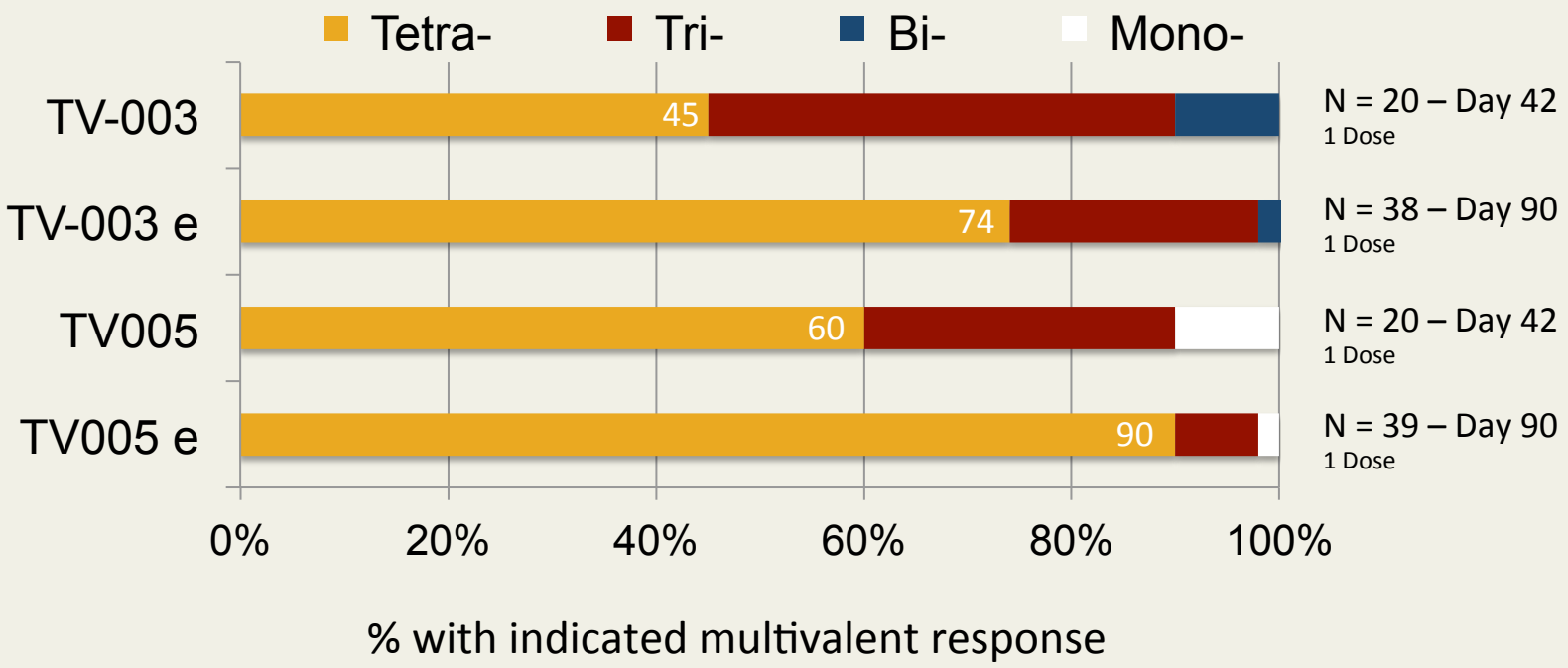
TV-003: 3, 3, 3, 3
TV-005: 3, 4, 3, 3

		% seroconverted ($PRNT_{50} \geq 10$)				Mean peak titer (GMT) ($PRNT_{50} \geq 10$)			
Vaccine	**N**	**DEN1**	**DEN2**	**DEN3**	**DEN4**	**DEN1**	**DEN2**	**DEN3**	**DEN4**
TV-003	**20**	**100**	**50**	**100**	**100**	**106**	**64**	**42**	**86**
TV-003 e	**38**	**92**	**76**	**97**	**100**	**63**	**40**	**85**	**151**
TV-005	**20**	**90**	**60**	**90**	**100**	**52**	**63**	**41**	**76**
TV005 e	**39**	**92**	**97**	**97**	**97**	**35**	**91**	**100**	**205**

TV003 neutralizing antibody response



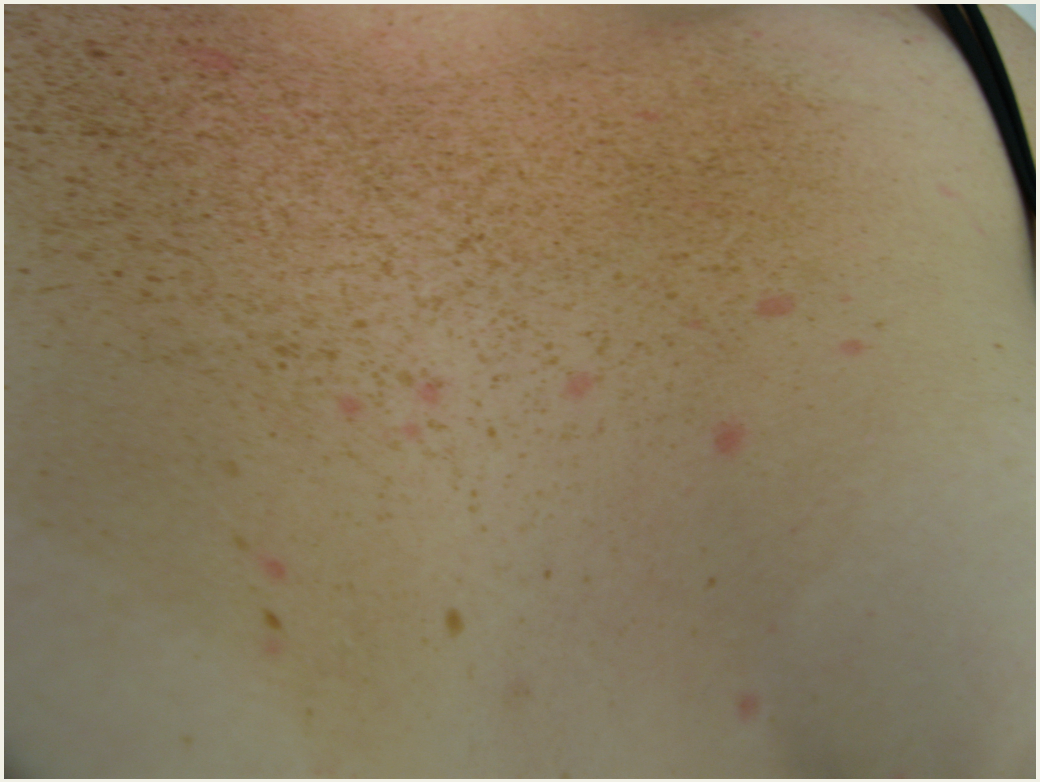
TV003 & TV005 neutralizing antibody response

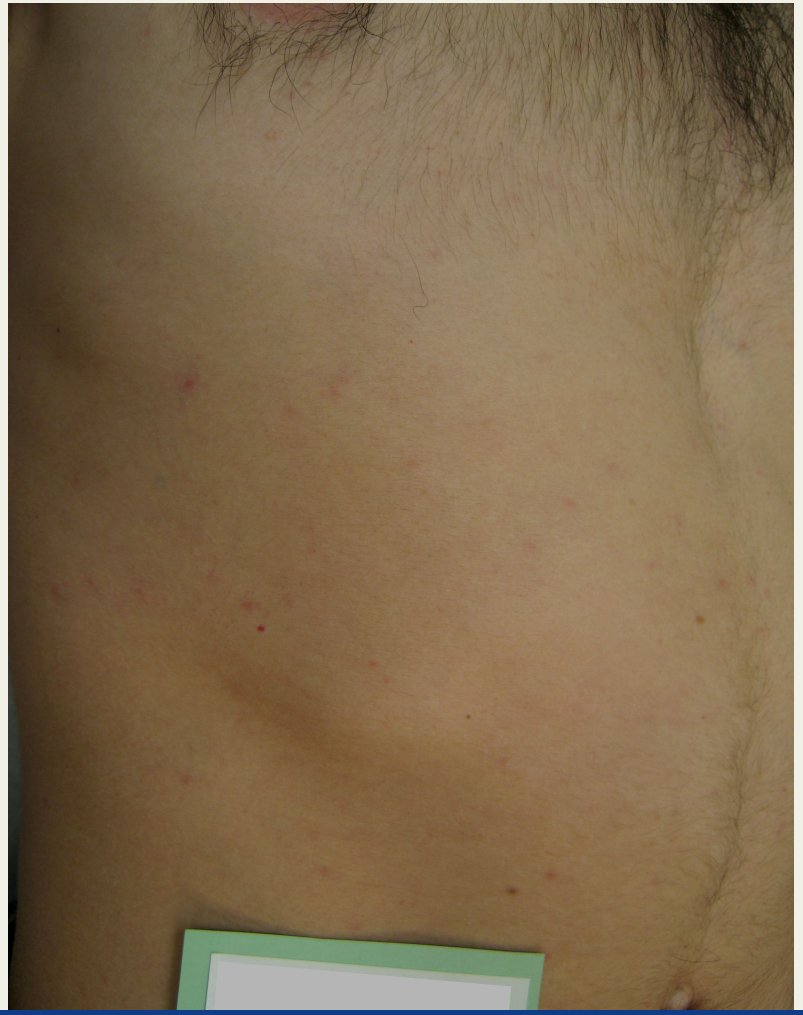


Clinical summary of adverse events

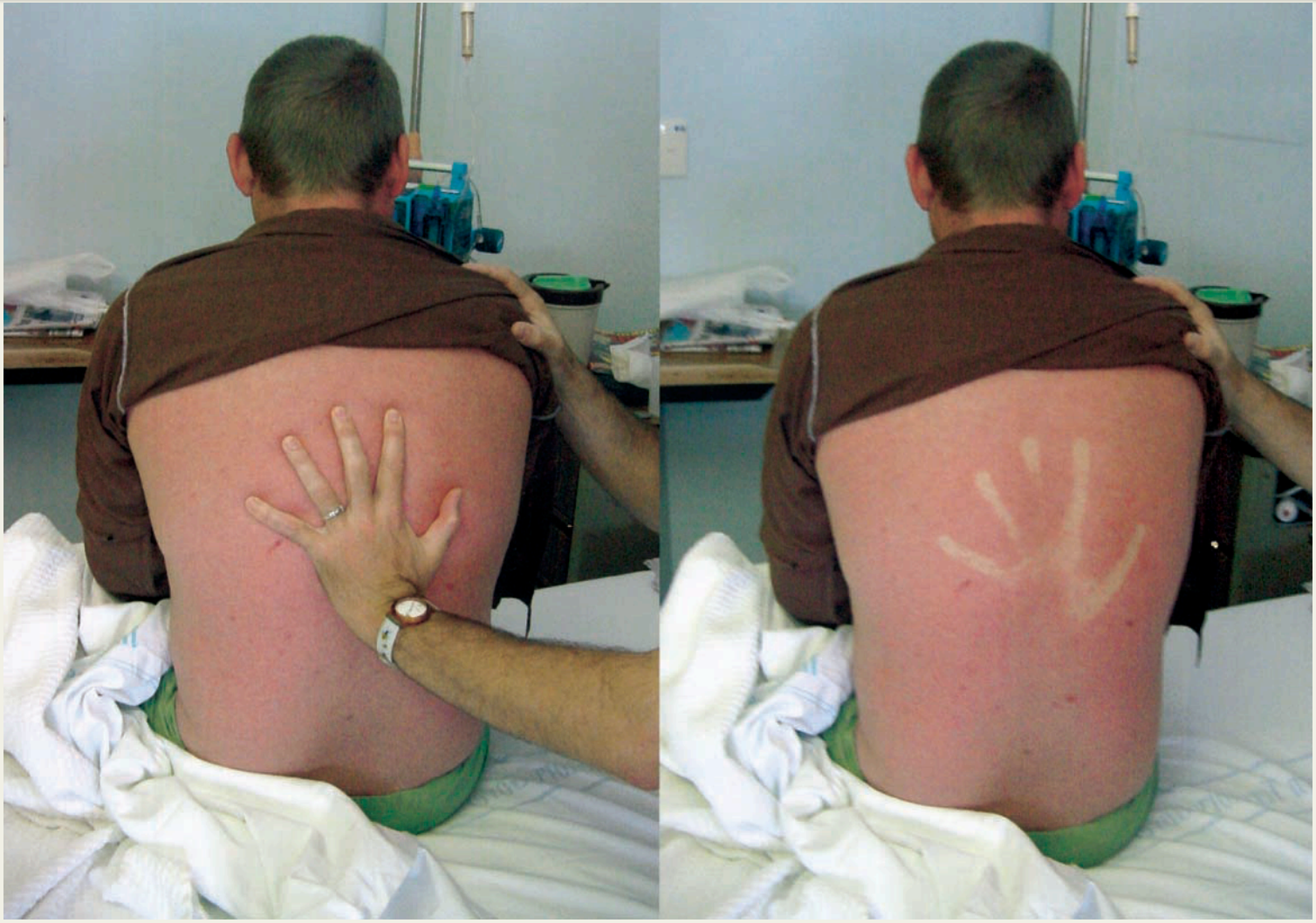
Adverse event	TV-003 (n=40)	Placebo (n=16)	p-value	TV-005 (n=40)	Placebo (n=16)	p-value
Injection site:						
Erythema	5.0%	6.3%	1.0000	2.5%	0.0%	1.0000
Pain	0.0%	6.3%	0.2857	2.5%	0%	1.0000
Tenderness	5.0%	0.0%	1.0000	0.0%	6.3%	0.2857
Induration	5.0%	0.0%	1.0000	2.5%	0.0%	1.0000
Systemic:						
Fever	0.0%	0.0%	n/a	2.5%	0.0%	1.0000
Headache	45%	25%	0.2300	57.5%	37.5%	0.2397
Rash	**55%**	**0.0%**	**< 0.0001**	**67.5%**	**0.0%**	**<0.0001**
Neutropenia[b]	2.5%	6.3%	0.4935	7.5%	0%	0.5498
Elevated ALT[c]	5.0%	0.0%	1.0000	5.0%	6.3%	1.0000
Myalgia	7.5%	6.3%	1.0000	10.0%	6.3%	1.0000
Arthralgia	0.0%	6.3%	0.2857	0.0%	0.0%	n/a
Retro-orbital pain	5.0%	0.0%	1.0000	7.5%	12.5%	0.6172
Fatigue	20.0%	0.0%	0.0892	32.5%	31.3%	1.0000
Photophobia	0.0%	0.0%	n/a	5.0%	6.3%	1.0000
Elevated PT	2.5%	6.3%	0.4935	5.0%	6.3%	1.0000
Elevated PTT	3.6%	12.5%	0.0779	0.0%	0.0%	n/a
Thrombocytopenia	0.0%	0.0%	n/a	0.0%	0.0%	n/a

Vaccine-associated rash - asymptomatic



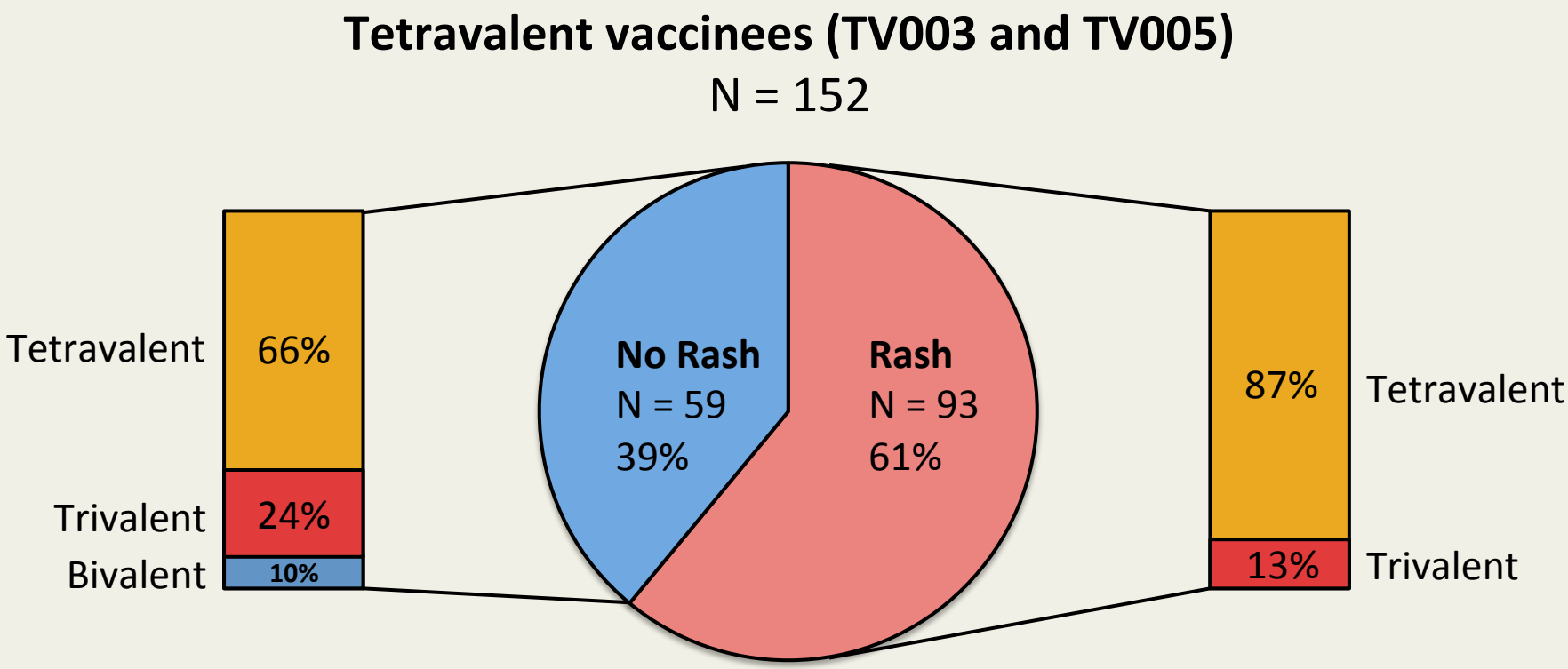


Wild-type dengue Rash



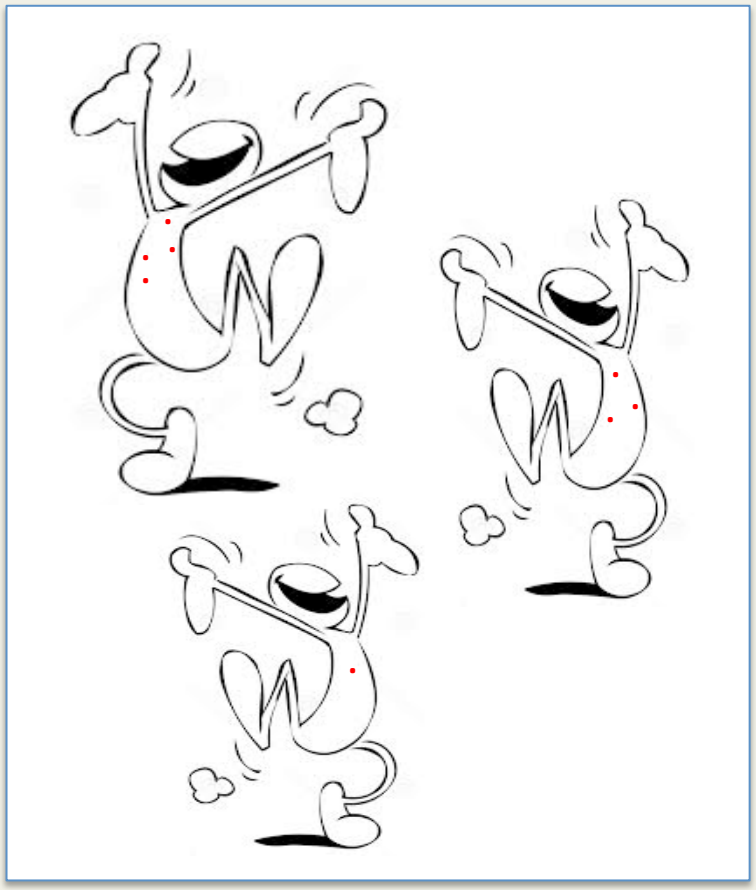
CID 2004: 38 (15 May)

Vaccine rash is predictive of a tetravalent antibody response



A vaccinee is statistically more likely to have a tetravalent antibody response if they present with a vaccine-associated rash (*P* = 0.002, Chi-square)

Vaccine rash is predictive of a tetravalent antibody response



Maybe it's a "happy" rash

Other vaccines with rash side-effects:

- MMR
- Varicella
- Zoster
- Yellow fever
- Japanese encephalitis

Dengue Disease

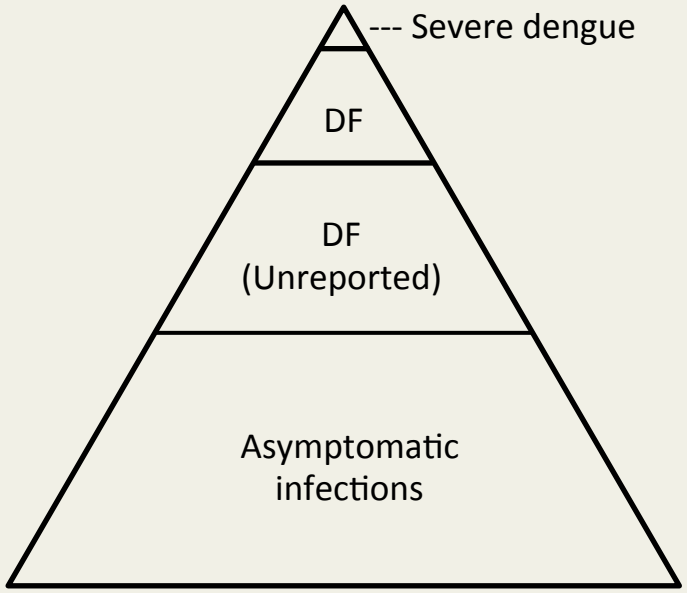
Dengue Fever

- **Fever**
  and
- **Headache**
- **Retro-orbital pain**
- **Myalgia**
- **Arthralgia**
- **Hemorrhage**
- **Rash**

- **Leukopenia**
- **Neutropenia**
- **Elevated ALT / AST**
- **Viremia**
- **Serum Antibodies**

Severe Dengue

- **Fever (2 – 7 days)**
  and
- **Thrombocytopenia**
  and
- **Petechial rash**
- **Bruising**
- **Bleeding**
- **Coagulopathy**
  and
- **Vascular leakage**
  - **Pleural effusion**
  - **Ascites**
- **Hemoconcentration**



Shock Syndrome (DSS):
- Hypotension
- Shock

One dose or two?

Clinical evaluation of NIAID TV-005
Two dose (6 months apart)
Neutralizing antibody: 1 - 3 months post vaccination

TV-005e		% seroconverted ($PRNT_{50} \geq 10$)				Mean peak titer (GMT)			
Vaccine	N	DEN1	DEN2	DEN3	DEN4	DEN1	DEN2	DEN3	DEN4
Dose 1	38	92	97	97	97	35	91	100	205
			6 months after Dose 1:			10	39	26	39
Dose 1 + 2	33	94	100	100	100	16	55	36	75
			Fold change after dose 2:			1.6x	1.4x	1.4x	1.9x

	First dose		Second dose	
Vaccine	Viremia	Rash	Viremia	Rash
TV-005	70%	68%	0%	0%

Nearly sterilizing immunity at 6 months post vaccination = minimal antibody boost

Live attenuated dengue vaccines

	Sanofi-Pasteur	Takeda	NIH
Doses	3	2	1
Potency (log_{10} per serotype)	5, 5, 5, 5	4, 4, 4, 5	3, 4, 3, 3
% tetravalent response (DEN-naïve subjects & SQ)	78%*	58%**	90%
CD8 T-cell epitopes	YFV	DEN2	DEN1, 3, 4
Clinical phase	3	2	2
Overall efficacy	30 – 61%	?	?

* Villar, *et al.* 2011. *Ped. Inf. Dis. J.* Oct 2013.
** Osorio, *et al., Lancet*, July 2014.

DENV challenge of vaccinees

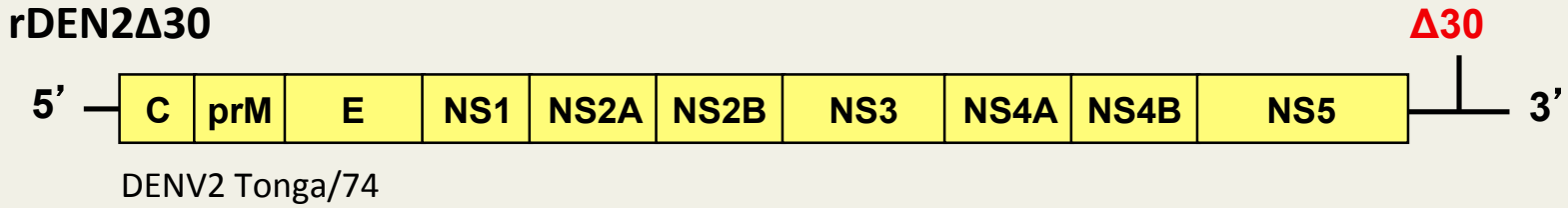
Dengue Vaccine Development at the LID

100's of virus constructs

Pre-clinical candidates (monkeys)	Phase I candidates (>700 subjects)	Phase I tetravalent (>175 subjects)	Phase II tetravalent
DEN1Δ30 DEN1/4 DEN1/4Δ30 DEN1/4Δ30(CME) DEN1mutF	DEN1Δ30	DEN1Δ30	**DEN1Δ30**
DEN2Δ30 DEN2Δ30-4995 DEN2/4 DEN2/4Δ30 DEN2/4Δ30(CME) DEN2-3'D4Δ30	DEN2/4Δ30 DEN2Δ30	DEN2/4Δ30	**DEN2/4Δ30**
DEN3Δ30 DEN3/4 DEN3/4Δ30 DEN3/4Δ30(CME) DEN3/4Δ30(Sri Lanka) DEN3Δ86 DEN3Δ30/31 DEN3-3'D4Δ30	DEN3/4Δ30 DEN3Δ30/31 DEN3-3'D4Δ30	DEN3Δ30/31 DEN3-3'D4Δ30	**DEN3Δ30/31**
DEN4Δ30 DEN4Δ30-200,201 DEN4Δ30-436-437 DEN4Δ30-4995	DEN4Δ30 DEN4Δ30-4995 DEN4Δ30-200,201	DEN4Δ30 DEN4Δ30-200,201	**DEN4Δ30**

Safe, highly infectious, balanced immune response

rDEN2Δ30



DEN2Δ30 developed as candidate DENV-2 vaccine

- Derived from Tonga/74: Isolated from DENV-2 outbreak that caused milder disease and lower levels of viremia
- Different genotype than DENV-2 in TV003
- Was less attenuated in rhesus macaques compared with rDEN2/4Δ30

DEN2Δ30 in healthy volunteers

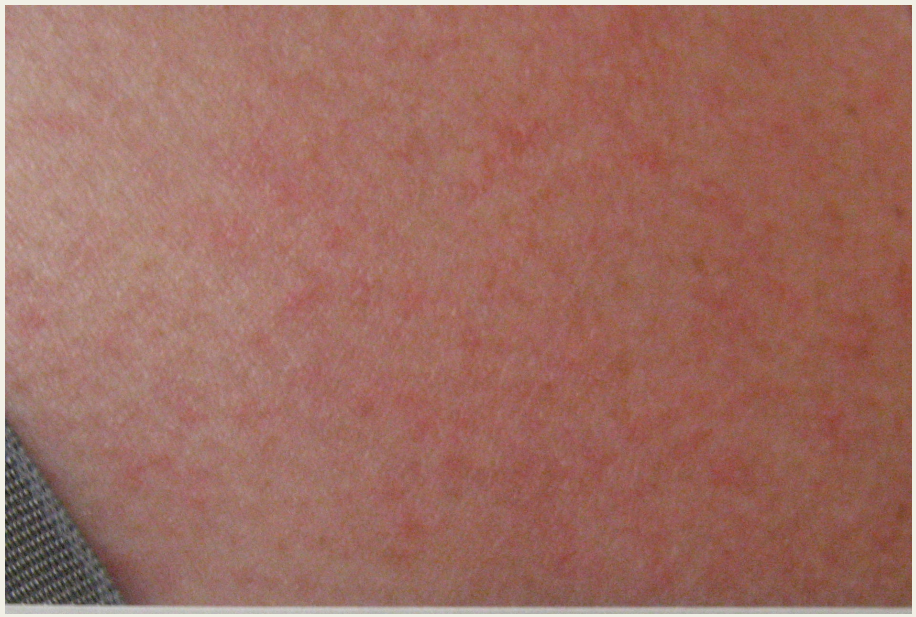
- 10 subjects received 10^3 pfu of rDEN2/4Δ30
- 10/10 subjects with viremia
- 8/10 subjects with rash
  - Character of rash different: more diffuse, pruritic
  - 50% moderate in intensity
- 4/10 subjects with neutropenia
  - Moderate in 2 subjects (ANC nadir = 592/mm^3 and 695/mm^3, both day 11)
  - Mild in 2 subjects (ANC nadir = 806/mm^3 and 961/mm^3, both day 11)
- **No subject developed fever, elevated LFTs, or signs vascular leak**

Vaccine Challenge Study

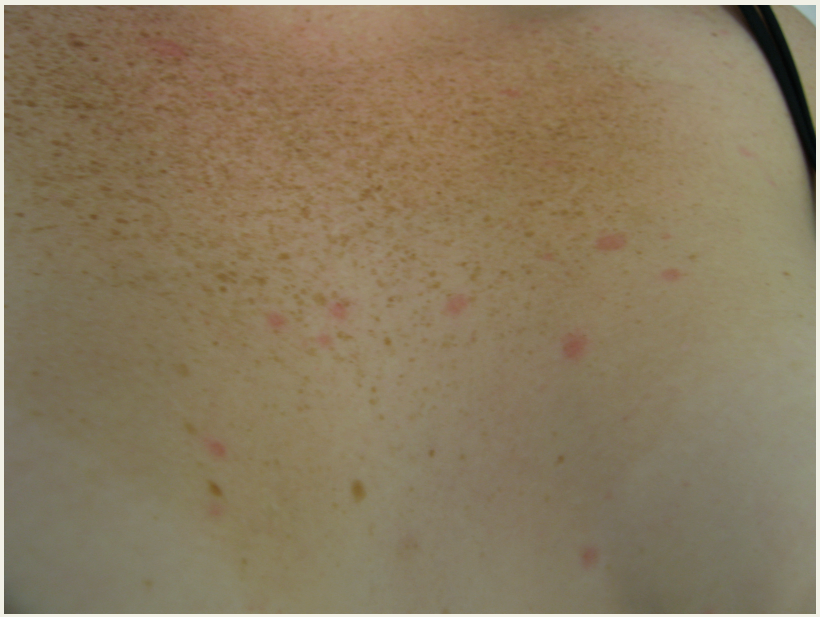
Adverse event	DEN2Δ30 (n=20)	Placebo (n=14)	p-value
Injection site:			
Erythema	0.0%	0.0%	n/a
Pain	0.0%	10.7%	0.1062
Tenderness	0.0%	3.6%	0.4828
Induration	0.0%	0.0%	n/a
Systemic:			
Fever	0.0%	0.0%	n/a
Headache	40.0%	21.4%	0.1613
Rash	**80.0%**	**0.0%**	**<0.0001**
Neutropenia	**26.7%**	**0.0%**	**0.0047**
Elevated ALT	3.3%	3.6%	1.0000
Myalgia	20.0%	7.1%	0.2555
Arthralgia	10.0%	0.0%	0.2377
Retro-orbital Pain	**30.0%**	**0.0%**	**0.0020**
Fatigue	26.7%	17.9%	0.5432
Prolonged PT	6.7%	14.3%	0.4155
Prolonged PTT	0.0%	7.1%	0.2287
Thrombocytopenia	6.7%	0.0%	0.4918

[A] CIR268 and CIR 287 combined

DEN2Δ30 rash



DEN2Δ30



Typical DENV vaccine rash

Viremia summary rDEN2Δ30

Virus	Dose (log_{10} PFU)	N	% with viremia	Mean peak titer ± SE (range)[1]	Mean day of onset of viremia ± SE	Mean # days of viremia ±SE
rDEN2Δ30	3	10	100[2]	2.5 ± 0.2[3] (1.5 - 3.3)	4.6 ± 0.4[3]	5.8 ± 0.6[3]
rDEN2/4Δ30	3	40	60[2]	0.5 ± 0.03[3] (0.5 - 1.2)	9.2 ± 0.6[3]	3.3± 0.6[3]

1. log_{10} PFU/mL
2. Statistically significant (p=0.02)
3. Statistically significant (α = 0.01)

Challenge strain vs. vaccine strain:

- Viremia 100-fold higher
- Onset of viremia earlier
- Duration of viremia longer

Evaluation of vaccine efficacy

- 48 subjects enrolled: 24 receive TV003 and 24 receive placebo as single dose
- 6 months later all 48 receive 10^3 PFU DEN2Δ30
  - Primary efficacy endpoint is protection against viremia with DEN2Δ30 (60% efficacy at a power of 0.8)
  - Secondary efficacy endpoints are protection against rash and neutropenia
- Samples collected every other day for 16 days then at days 21, 28, 56, 90, and 150 post vaccination and same schedule post challenge

DENV-2 Vaccine Challenge Study

Viremia post-challenge with DEN2Δ30						
Cohort	N	Frequency of viremia	Mean peak Viremia*	Viremia range*	Mean day of onset	Mean duration (days)
Placebo	19	100%	2.3 ± 0.1	1.5 – 2.9	4.9 ± 0.6	5.4 ± 0.5
TV-003	21	0%	n/a	n/a	n/a	n/a

Rash presentation post-challenge with DEN2Δ30					
Cohort	N	Frequency of rash	Mean day of onset	Mean duration (days)	Intensity
Placebo	19	84%	9.3	7.6	32% moderate 68% mild
TV-003	21	0%	n/a	n/a	n/a

- **TV-003 provides 100% efficacy against DEN2 challenge viremia and rash**

DENV-2 Vaccine Challenge Study

		% seroconverted ($PRNT_{50} \geq 10$)				Mean peak titer (GMT)			
Vaccine	**N**	**DEN1**	**DEN2**	**DEN3**	**DEN4**	**DEN1**	**DEN2**	**DEN3**	**DEN4**
TV-003	24	92	100	100	100	47	84	152	270
		92% tetravalent response							
Post-Chall.	21						93		

Summary of NIAID Vaccine

- To date:
  - 124 naïve subjects have received TV003
  - 60 naïve subjects have received TV005
- Both admixtures are safe and well-tolerated
  - Both produce similar mild vaccine rash
  - All serotypes replicate following inoculation
- Frequency of tetravalent seroconversion is highest for TV-005 admixture (>90%)
- Induces both neutralizing and T-cell immunity directed towards DENV
- Due to induction of nearly sterilizing immunity, booster dose is not necessary in the short term
- This is a live-attenuated single dose vaccine

Licensing partners

- Merck & Co., USA
- Butantan Foundation, Sao Paulo, Brazil
- Panacea Biotec, New Delhi, India
- Vabiotech, Hanoi, Vietnam
- Additional manufacturers in India (pending)
- Additional manufacturer in Thailand (pending)
- GSK (inactivated vaccine application)